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AMERICAN FERN JOURNAL

QUARTERLY JOURNAL OF THE AMERICAN FERN SOCIETY

VOLUME 106 NUMBER 3 JULY-SEPTEMBER 2016

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American Fern Journal 106(3):153–160 (2016)
Published on 19 October 2016

Seasonal Changes in Starch Content in Trophopods of *Matteuccia struthiopteris*

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Abstract.—Trophopods are modified stipe bases that function as starch-storage organs in a wide variety of mainly temperate ferns. Ever since they were first observed, the presence of trophopods has been explained by reference to seasonality and they have been assumed to provide nutrition for the rapidly expanding fern leaves in spring. We present the results of an analysis of the annual variation in starch content in *Matteuccia struthiopteris* (L.) Todaro, cultivated in the Leiden botanical garden. Our results show a distinct seasonal variation in starch content, suggesting that the presence of trophopods is indeed functionally linked to seasonality, but we do not find a decrease in starch content corresponding to the period of leaf expansion that would clearly link the depletion of stored starch to the expansion of new leaves.

Key Words.—fern leaf expansion, overwintering buds, petiole, seasonality

The term "trophopod" was coined by Wagner and Johnson (1983), to denote "a petiole base that is specialized to accumulate food and to persist as a storage organ…". The first accurate description of this structure, however, is given by Eaton (1879) in his description of *Aspidium goldianum* Hooker. He describes the basic elements that characterize these structures: the thickness, the continuity with the rhizome, the persistence, and the starchy contents:

"The root-stock is creeping or ascending, several inches long, and nearly an inch thick. This thickness is made up, in considerable part, by the adherent bases of old stalks; the stalks being perfectly continuous with the root-stock, and so much crowded as to overlap each other. When fresh the root-stock is fleshy, and a longitudinal section of it shows that its substance passes so gradually into that of the stalk-bases, that no point of separation or distinction between the two can be selected. (…) The parenchymatous portion of the root-stock is loaded with starch in very minute grains, as may be easily proved by adding a drop of alcoholic solution of iodine to a thin slice of the root-stock

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corresponding author: peter.hovenkamp@naturalis.nl placed under a microscope, when the grains will be presently seen to turn blue, the recognized sign of starch."

It is not surprising that he then correlated the presence of starch-containing structures with the annual growth of a new crown of leaves: "This abundance of nutritive material in the root-stock enables it to send up a fine circle of large leaves in the proper season of the year."

Eaton's observations have been confirmed in a number of studies, and over the course of the following century, they have been supplemented by observations of trophopods in a number of different species. Wagner and Johnson (1983) observed that trophopods are widespread in North American ferns, and that there is a correlation both with taxonomy and with ecology, as they are absent in several large taxonomic groups, as well as in obligatory rock-dwelling species. In passing, they also note that trophopods occur both in seasonal and in wintergreen taxa. In a more detailed study, Johnson (1986) noted that there may also be a correlation with climate, trophopods being absent in the predominantly tropical genus *Diplazium*, and present in related temperate groups. This conjecture is presented as fact by Lellinger (2002), who defined a trophopod as: "the enlarged, persistent, basal portion of a stipe that functions as storage organ, especially through the cold months, found in some ferns of temperate regions." Since then, however, anecdotal observations suggest that trophopods are also found in ferns from tropical climates. Vasco *et al.* (2013) report starch-filled trophopods in two tropical species of *Diplazium* in Central America. Hovenkamp (irregularly updated), in a study of the genera *Diplazium* and *Athyrium* on Mount Kinabalu (Malaysia, Sabah, Borneo) found that trophopods were regularly present in the *Athyrium* species growing on the temperate high slopes of the mountain, but also sporadically found in species from the closely related genus *Diplazium* growing on the warm-tropical lower slopes. Sundue and Rothfels (2014) state that trophopods are "perhaps best developed among predominantly temperate groups", but are "also present among sub-tropical and tropical species", and summarize that the relative prevalence of trophopods in tropical and temperate plants should be examined more thoroughly. An obvious functional interpretation for a higher prevalence of trophopods in temperate, seasonal, areas would be, as noted already by Eaton (1879), that they play a role in supporting the rapid expansion of new fern leaves in spring.

Here we attempt to confirm this functional explanation by examining the seasonal changes of the starch contents in *Matteuccia struthiopteris* (L.) Todaro (Fig. 1), which is native to the colder temperate regions of the Northern hemisphere (Odland *et al.*, 2004), where it may form large stands at least partly by vegetative reproduction. It is a deciduous plant, and expansion of the leaves in spring occurs in little more than four weeks (Von Aderkas and Green, 1986). In addition to leaves, in the course of the growing season, cataphylls, abortive leaves that consist of little more than the trophopods, develop as well as sporophylls, non-assimilating spore-producing leaves. All leaves have very distinct trophopods at the base of the stalk, which persist for an unknown number of years after the leaves are shed (Fig. 2).

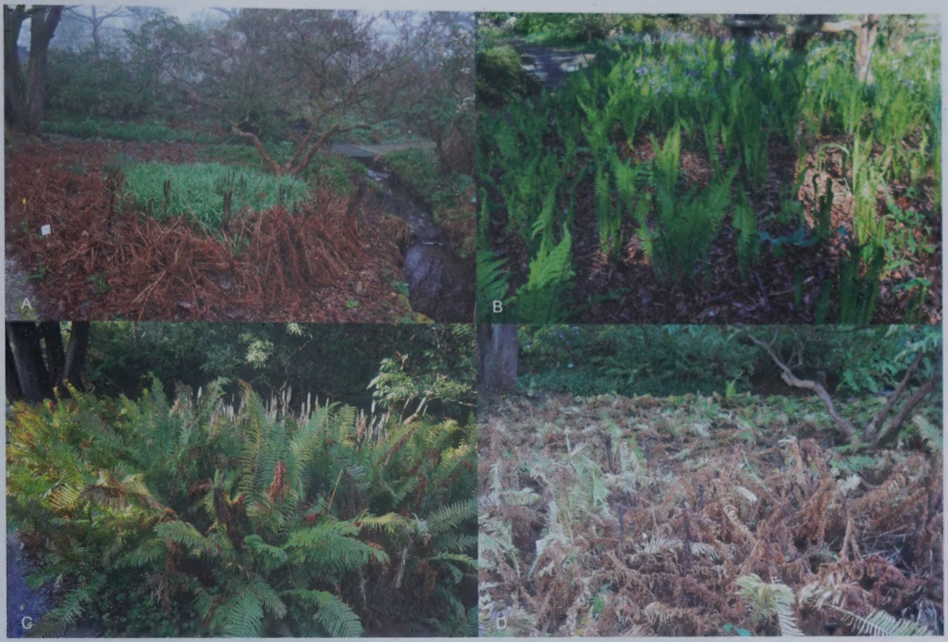

FIG. 1. Seasonal changes in the aspect of the sampled *Matteuccia* patch in the Hortus botanicus Leiden in the sampled period. A – March 14, B – April 16, C – September 17, D – October 20.

As *M. struthiopteris* is a source for edible fiddleheads, which are commercially harvested from the wild (DeLong and Prange, 2008), some experiments have studied the effect of complete removal of croziers (fiddlehead harvest). Bergeron and Lapointe (2001) found that starch was the main form of carbohydrate reserve in trophopods when measured at the end of the growing season, and that complete defoliation early in the growing season could reduce this starch content by 88%.

MATERIALS AND METHODS

For a study site, we chose a patch in the Leiden botanical garden, where several hundreds of individual plants (crowns) occur on a surface of c. 25 m^2. As *Matteuccia* readily spreads by way of underground stolons, and the source of the original plant material could not be retrieved, the entire patch may well represent a single clone (Grzybowski and Kruk 2015). We sampled at roughly monthly intervals over the period February 2014 – February 2015, thus covering an entire growing season. Leaf expansion took place in late March and April; leaf dieback started at the end of September and was complete by the end of October (Fig. 1). From five separate crowns, we took samples of three or four trophopods on different levels of the crown, which we labeled L for trophopods from low down on the crown, M for those from middle level, H for the upper level and G for the uppermost, fresh, green ones. In total, 233

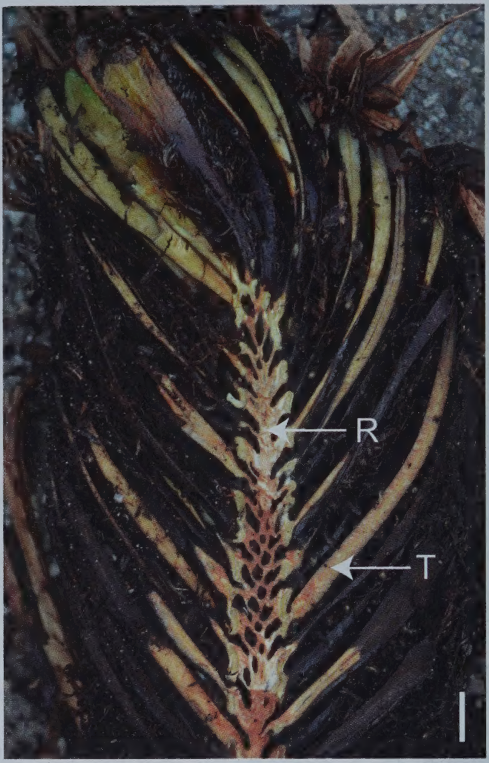

FIG. 2. Approximately medial longitudinal section through a crown of *Matteuccia struthiopteris*. R: rhizome, T: trophopod, scale bar 1 cm.

separate samples were analyzed because not all sampled crowns were robust enough to support the removal of four parts at different levels. Our intention was that by sampling in this way, the samples from the middle and upper level would correspond to the trophopods remaining from leaves that had abscised the previous year, lower-level trophopods to those from earlier years, and the uppermost ones to represent the metabolically most active part of the plant. In the months February to September the parts marked G corresponded to the dormant buds formed the previous year and the expanded leaves, in the months October to January these were the dormant buds formed during the sampling period. We did not distinguish between trophopods of foliage leaves and cataphylls, and whenever possible, we tried to avoid sampling from fertile leaves that are non-assimilating and therefore can be expected to have a different starch metabolism. The total number of well-developed crowns in the patch did not allow us to sample entire crowns destructively. Accordingly, some crowns may have been sampled repeatedly at different times, and the assignment of trophopods to categories L, M or H may have had some degree of arbitrariness.

For the analysis, the collected tissues were stored at –80°C until they were ground using a pestle and mortar under liquid nitrogen. The ground tissues were then transferred to 2 ml centrifuge tubes and placed in a freeze-dryer for 48 hours. For each sample, 30 mg dry tissue was accurately weighted and analyzed using the protocol of Smith and Zeeman (2006), using the Glucose (HK) Assay Kit obtained from Sigma-Aldrich. The data are available in the Dryad repository: doi:10.5061/dryad.3113c.

Results

Starch content of trophopods from all different levels of the rhizome increases roughly twofold over the summer period, when the leaves are metabolically active (Figs. 3, 4). There is a slight decrease between the values for March and April, but the major decrease occurs immediately after the maximum value has been reached in September and October. This is even more clearly visible in the green parts (Fig. 3). Here, the values for February to September refer to the overwintering buds and their expansion into new leaves, and the values for October to January show the changes occurring in the newly formed buds. The most dramatic decrease in starch content thus does not take place when the buds expand into new leaves (March–April), but occurs in the newly formed buds just after they have been formed on the plant.

Discussion

Variation in starch content on a single rhizome.—The starch content of trophopods at all four levels on the rhizome shows a clearly similar pattern of variation over the year (Fig. 3). Even taking into account that without destructive sampling of entire crowns, we could not confidently estimate the ages of all the sampled trophopods, and that the labeling of trophopods as from different levels may be partly erroneous, the general trend is clear. We expect therefore that this uncertainty does not affect our main results seriously. Our results then show that all trophopods, the older ones as well as the recently formed ones, are involved in the metabolism of the plant.

Seasonal variation.—Seasonal variation in starch storage shows a clear pattern (Figs. 3, 4). Over the growing season, there is an increase in starch content that amounts to a doubling or more, leading to starch contents at the end of the season that attain levels of around 40% of dry weight of the trophopod, even slightly higher for the newly formed dormant buds. These values are somewhat higher than the levels found at the end of the season in a more natural setting (Bergeron and Lapointe, 2001), which may be due either to differences in the analytical protocol, or to the better circumstances for the plants provided in our garden setting. It has been suggested that in wintergreen ferns, old leaves act as storage organs to support the emergence of new leaves (e.g., Landi *et al.*, 2014), and based on this, Eaton's (1879) expectation seems reasonable that in winter-dormant ferns new growth would be supported by the starch stored in trophopods. However, in our data leaf expansion is

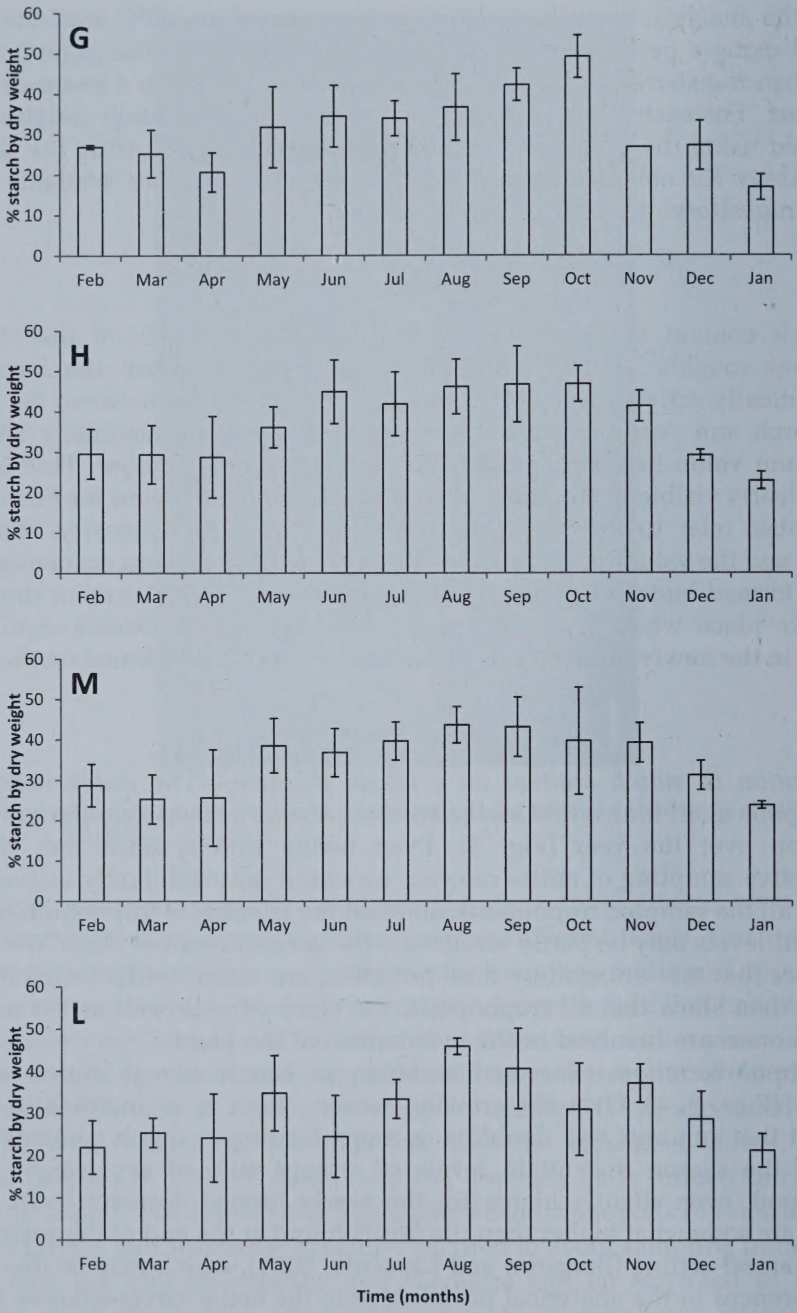

FIG 3. Seasonal changes at different levels (L=Low, M=Middle, H=High, G=Green) on the rhizome. Error bars correspond to one standard deviation in both directions.

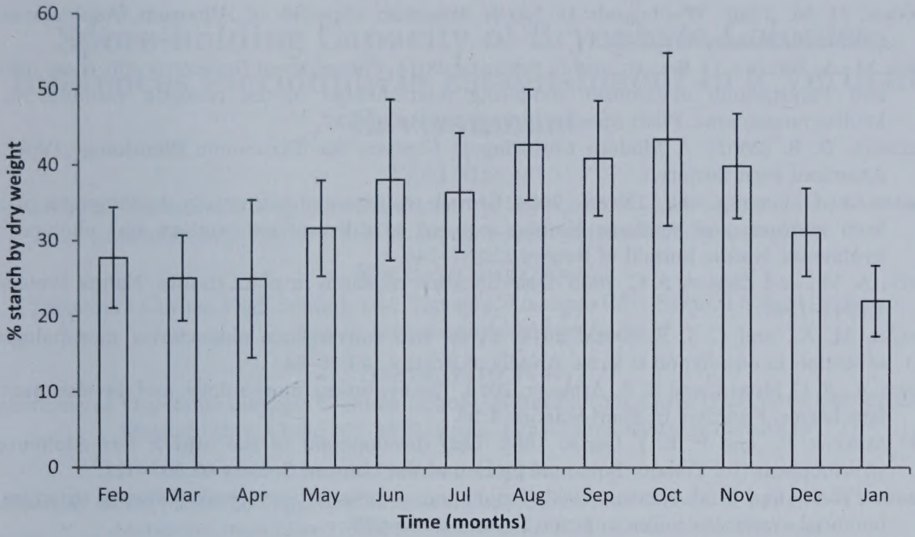

FIG 4. Seasonal changes in starch content pooled for all levels on the rhizome. Error bars correspond to one standard deviation in both directions.

unconnected to starch depletion. The main depletion of the starch reserves in trophopods of *Matteuccia* does not take place when the new leaves expand in spring, but immediately after the maximum level has been reached and the leaves have died back in autumn. At that time, there is no obvious destination for the carbohydrates that are thus released, which suggests that they are stored in a different location, or in a form that is not detected by Smith and Zeeman's (2006) protocol for starch analysis. In view of the very limited volume of the actual rhizome of a crown, compared to the aggregated volume of the trophopods, and the anatomical continuity between these organs, we think that storage of starch in the rhizome proper is not a likely explanation of the observed depletion of the trophopods. Thus, while distinct seasonal variation in starch contents suggests a functional link between the metabolism of the trophopod and the seasonality of the plant, the exact nature of this link requires further investigation into alternative modes of storage for carbohydrates through the dormant season.

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American Fern Journal 106(3):161–170 (2016)
Published on 19 October 2016

Spore-holding Capacity of Bryophyte Colonies Influences Pteridophyte Establishment in a Vertical Environment

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Abstract.—Gametophytes of epiphytic and epipetric pteridophytes occur frequently in bryophyte colonies; however, little is known about the influence of bryophytes on pteridophyte establishment. In the present study, we describe how the spore-holding capacity of bryophyte colonies contributes to the retention of pteridophyte spores in a vertical environment. Lycophytes did not grow at the sampling site; hence, we used *Lycopodium* spores to distinguish from any other pteridophyte spores that might have already been present in the wild. We dispersed aliquots of approximately 500,000 spores on vertically suspended bryophyte (both mosses and liverworts) colonies (5 cm × 5 cm) attached to ceramic plates. The bryophyte colonies included ten species (seven mosses and three liverworts), which have different characteristics in colony height, leaf length, and dry weight. Mudstone blocks and ceramic plates were used as control surfaces (5 cm × 5 cm). After simulated rainfall, we counted the number of spores retained in the colonies and controls. We found that the bryophyte colonies retained a significantly higher percentage of *Lycopodium* spores (90.7±7.4%) than the controls (ceramic plate: 7.5±20.9%; mudstone: 39.7±20.6%). The average values of retained spores within the bryophyte colonies correlated with their logarithmic values of dry weight per square centimeter ($R^2 = 0.62$, $P < 0.05$). Leaf thickness and stem diameter were slightly different across species. Thus, dry weight value of bryophyte colony per unit area is very likely a simple function of number of stem-and-leaf units, which correlate closely with the surface area. Bryophyte colonies that have high surface area on which pteridophyte spores might be retained within a colony might provide a suitable environment for spore adherence in epiphytic and epipetric habitats.

Key Words.—Epiphytes, pteridophytes, gametophytes, epipetric plants, rainfall, spore adherence

Bryophytes are the most common components of epiphyte communities and have a larger biomass than epiphytic vascular plants in Neotropical forests (Nadkarni, 1984). By extending their protonemata after spore germination (Iwatsuki, 2001), bryophyte gametophytes can cling to uneven surfaces, allowing many bryophyte species to establish colonies on the vertical surfaces of rocks and tree trunks (Smith, 1982). They can survive in these soil-poor, dry habitats due to their poikilohydric nature (i.e., capacity to reestablish metabolic function following desiccation and rehydration).

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Bryophyte colonies provide suitable environments for the establishment of seed plants (Nakamura, 1992; Saxena and Harinder, 2004; Yatoh, 1963), since their ability to retain water (Barkman, 1958; Cross, 1981; Pócs, 1980) facilitates seed germination and the initial growth of seedlings. In addition, existing hypotheses suggest that bryophyte bodies function as anchors to hold the seeds of plants in place on the tree trunks that characterize epiphytic habitats (Ingram and Nadkarni, 1993).

In addition to seed plants, pteridophytes (here inclusive of Lycophytes) are also frequently found on vertical environments. Previous studies have shown that the existence of bryophyte colonies on tree trunks and vertical rock cliffs facilitates pteridophyte establishments. For example, Mizuno *et al.* (2012b) showed that the young sporophytes of epipetric *Crypsinus hastatus* occurred more frequently on bryophyte colonies than on bare surface of rocks, likely because bryophyte colonies prevent the washout of its spores by rainfall. However, the distribution density of gametophytes of epipetric pteridophyte differed across bryophyte colony structures (Mizuno *et al.,* 2012a). Thus, the spore-holding capacity of bryophyte colonies might depend on bryophyte shape and structure, thereby affecting pteridophyte establishment on vertical habitats. Clarifying the relationship between spore-holding capacity and pteridophyte establishment might provide an insight into the maintenance of epiphytic and epipetric plant diversity.

In this study, we performed a laboratory experiment to clarify the influence of bryophyte colony density, colony height, and leaf length on the capacity of bryophyte colonies to retain pteridophyte spores.

Materials and Methods

Sampling of bryophyte colonies.—We conducted sampling in Kimitsu City (Chiba Prefecture, eastern Japan; 35°12′N, 140°2′E) and in Owase City (southern Mie Prefecture, central Japan; 34°4′N, 136°10′E). We collected 5 colonies each of 10 bryophyte species with different colony characteristics, focusing on colony height and leaf length (Table 1). No species that we collected have any lamellae in which pteridophyte spore might be retained.

We collected bryophyte colonies from rock cliffs. We used a metallic spatula to remove each colony, while ensuring to retain the colony structure. We placed each colony into separate zip-lock bags and stored them in a cooler to maintain moisture levels and colony structure during transfer to the laboratory.

We then measured colony height, leaf length, and dry weight per square centimeter of a colony. To measure the colony height, we randomly inserted a needle into bryophyte colonies and measured the length of the inserted needle. The leaf length values were measured using magnified images. We first

TABLE 1. Percentage of retained spores, dry weight per square centimeter, colony height, and leaf length of the 10 bryophyte species used in the study.

Bryophyte species	Moss or liverwort	Percentage of retained spores (%)	Colony height (mm)	Leaf length (mm)	Dry weight/ area (mg/cm^2)
Control 1 (Ceramic plate)	–	7.5 ± 20.9	–	–	–
Control 2 (Mudstone block)	–	39.7 ± 20.6	–	–	–
Brachythecium buchananii	Moss	92.2 ± 6.9	10.9 ± 3.0	2.0 ± 0.4	50.4 ± 9.8
Ctenidium capillifolium	Moss	93.0 ± 7.2	5.8 ± 1.0	1.1 ± 0.2	35.9 ± 3.8
Entodon challengeri	Moss	94.2 ± 4.9	9.7 ±1.0	1.6 ± 0.2	28.7 ± 2.8
Hypnum oldhamii	Moss	86.7 ± 9.3	6.2 ± 1.0	1.3 ± 0.1	13.9 ± 6.0
Jungermannia granulata	Liverwort	85.6 ± 8.1	3.7 ± 0.9	1.2 ± 0.2	14.8 ± 4.5
Jungermannia truncata	Liverwort	92.3 ± 7.2	9.2 ± 1.2	1.2 ± 0.2	82.2 ± 15.4
Kurzia makinoana	Liverwort	93.6 ± 6.0	13.2 ± 1.2	0.2 ± 0.0	39.7 ± 7.2
Leucobryum bowringii	Moss	92.5 ± 7.7	12.3 ± 1.4	4.3 ± 1.3	56.3 ± 20.2
Plagiomnium acutum	Moss	95.2 ± 5.0	14.7 ± 2.0	2.4 ± 0.3	51.7 ± 11.1
Thuidium cymbifolium	Moss	81.4 ± 9.8	10.8 ± 2.6	0.2 ± 0.0	14.8 ± 3.0

obtained a picture of the magnified bryophyte leaves and 1 mm of square grid, and then measured the length of bryophyte leaves and the square grid by using slide caliper and calculated the actual leaf length. Measurements of both colony height and leaf length were repeated 20 times to obtain the average values. Dry weight was measured using an electric balance "METTLER AE 240" (METTLER TOLEDO, Tokyo, Japan). We first sampled bryophyte colony into a 1 cm × 1 cm square. Next, we dried the colonies using a desiccator at 70°C for 24 h. We measured 5 samples of dry weight for each species and calculated the average density of colonies.

Setting of bryophyte colonies to ceramic plates.—We divided each bryophyte colony into the size of 5 cm × 5 cm by using a razor knife and bound them to 5 cm × 5 cm ceramic plates "BASEA, PN:AP01MN03UF" (TOTO LTD., Fukuoka, JAPAN) with nylon thread (diameter = 0.1 mm; Fig. 1). Other than creating colonies of a specific size, bryophyte colonies were not manipulated (i.e., trimming or cutting in between the shoots). Five sets of bryophyte colonies were prepared in total and kept moist in a refrigerator until the onset of the experiment. As controls, we prepared 5 empty ceramic plates. We also collected 5 mudstone blocks, which were inhabited by *Crypsinus hastatus* on the surface, from a cliff in Kimitsu City. We cleaned the surface and trimmed them into 5 cm × 5 cm plates, the same size as the ceramic plates.

Spore suspension.—To determine spore number, we prepared a *Lycopodium* spore suspension that contained a known quantity of spores. Because lycophytes did not grow at the sampling site, we used spores (particle size, approximately 28 μm) from "Sporopollenin *Lycopodium*" (Polyscience, Inc.; Warrington, PA, USA) to distinguish the added spores from any that might have already been present in the wild. We then prepared an aqueous solution of glycerol with a specific gravity (1.08) similar to that of spores to suspend them within the solution. We counted the number of *Lycopodium* spores in the solution by using a Thoma cell-counting chamber (Erma, Inc., Tokyo, JAPAN)

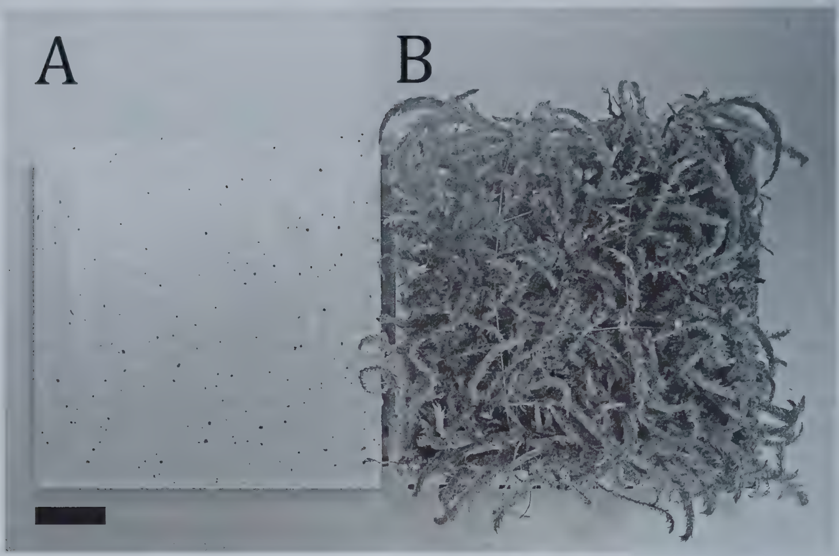

FIG. 1. A representative ceramic plate used to attach the bryophyte colonies (A), and a bryophyte colony that was formed and attached onto a ceramic plate by using a nylon thread (B). Bar: 10 mm.

to maintain spore density at 500,000 spores per milliliter solution. We heated the solution at 90°C for 30 min to prevent germination during storage and the subsequent experiment. We observed the spore suspension under light microscope to avoid heat-induced changes to spore structure after heating.

Simulated rain and measures of spore retention.—To add *Lycopodium* spores, we first dipped each bryophyte colony and controls (mudstone and ceramic plate) in water and drained them. Next, we used a micropipette to place 200 μL of the spore suspension onto 5 different positions on each colony surface (in the center and 1 cm from each of the 4 corners) to widely disperse the spores into the colonies and waited for the suspension penetrate into the colonies. In total, 1 mL of spore suspension (containing approximately 500,000 spores) was dispersed onto each colony (Fig. 2, Step1). Next, the colonies and controls were vertically affixed to the funnel of the equipment (Fig. 2, Step 2). The equipment consisted of a plastic funnel and cylinder. The funnel diameter and height were 6.5 cm and 7.0 cm, respectively. The cylinder diameter, height, and volume were 2.5 cm, 17.5 cm, and 50 mL, respectively. The funnel was placed on top of the cylinder and connected to each other via a hollowed-out rubber plug. The cylinder bottom was then anchored to the ground. Any spores that were present in the runoff produced when the colony and plates were attached were collected in the cylinder below the funnel. We showered tap water uniformly into the funnel from a height of 8 m above the equipment to simulate rainfall. We turned off the water when the measuring cylinder was filled to 10 mL (Fig. 2, Step 3), the equivalent of 20 mm/h precipitation. We then poured 10 mL of liquid into a centrifuge tube (Fig. 2, Step 4) and centrifuged for 3 min at 1,500 rpm (Fig. 2, Step 5). After the supernatant was

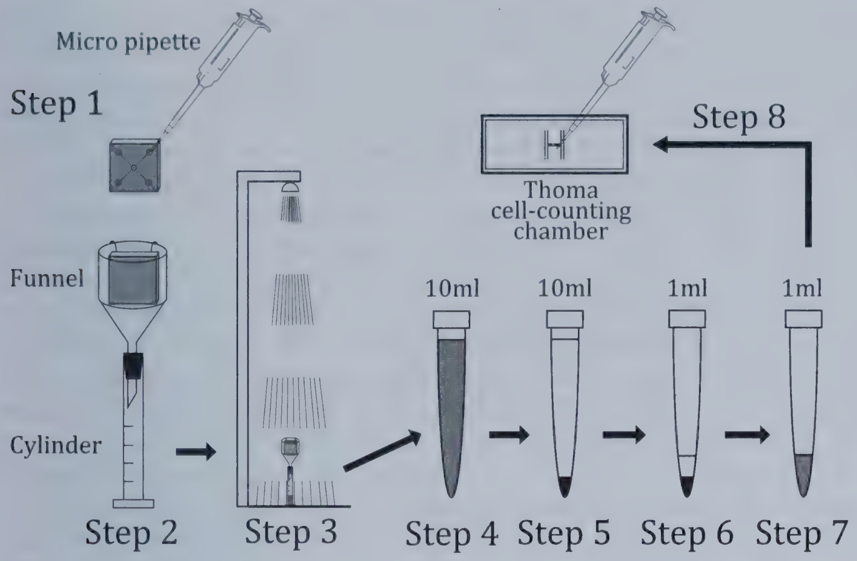

FIG. 2. Schematic of the study method. Step 1: Dispersal of 1 mL of spore suspension (containing approximately 500,000 spores) onto the bryophyte colony. Step 2: Attachment of bryophyte colonies and controls (mudstone and ceramic plate) to the plastic funnel of the experimental equipment. Step 3: Shower of tap water into the funnel from 8 m above. Step 4: The 10 mL of liquid was poured into a centrifuge tube. Step 5: Centrifugation for 3 min at 1,500 rpm. Step 6: Discarded 9 mL of supernatant. Step 7: Resuspension of spore pellets. Step 8: Next, 0.1 μL of the resuspension was poured into a Thoma cell-counting chamber, and the number of spores was counted under a light microscope.

discarded (9 mL; Fig. 2, Step 6), we resuspended the pellet (Fig. 2, Step 7) in 1 mL of solution. Next, we placed the resuspended solution into a Thoma cell-counting chamber by using a micropipette to count the number of *Lycopodium* spores in 0.1 μL of resuspended solution under a light microscope (Fig. 2, Step 8). The spores that might have already been present in the wild were distinguished from the added *Lycopodium* spores on the basis of the shape and ornamentation and excluded from the counting. We repeated the procedure (step 8) 20 times to obtain an average number of spores in the resuspended solution. Steps 1 to 8 were repeated 5 times for each bryophyte species and control. In all, we conducted 100 spore counts for each species and then estimated the percentage of spores that remained on the bryophyte colony by subtraction. We did not reuse the bryophyte colony once the spore suspension was dispersed.

Statistical analyses.—Significant differences in spore-holding capacity among each bryophyte species was determined by conducting multiple comparisons. First, we performed the Shapiro–Wilk test to test the normality of the data set of the number of counted spores. Because the normality was rejected by the test ($P < 0.05$), we used the Kruskal–Wallis nonparametric ANOVA followed by the Steel–Dwass nonparametric multiple comparison test. If the Kruskal–Wallis test showed significant results, Steel–Dwass multiple comparisons were performed. Next, we performed linear regression in order to test for linear relationships between each specific bryophyte trait and spore retention. Because the number of retained spores and values for each bryophyte trait were obtained separately and were not a pair of data points, we used average values for both these variables in the linear regression analysis. The average values of each bryophyte trait were log-transformed because the trends between the average percentage of retained spores and average values of bryophyte traits such as dry weight, colony height, and leaf length were curvilinear. These transformed values showed normal distribution. The average values of spore-holding capacity were arcsine-transformed to ensure normality before linear regression, because the values of neither non-transformed nor log-transformed average spore-holding capacity of each species showed normal distribution. The arcsine-transformed values of average spore-holding capacity and log-transformed values of bryophyte traits were used for linear regression analysis. Pearson's correlation coefficients (R^2) and significance (P-value) were calculated to identify which traits of bryophytes are strongly correlated with spore retention by using the average values of each bryophyte trait and average percentage of retained spores. The results of all statistical tests were considered significant when $P < 0.05$. Statistical analyses were performed using "R Statistical Environment (Ver. 3.2.2)" software.

RESULTS

The result of Kruskal–Wallis nonparametric ANOVA was significant among the 12 subsets (containing 10 bryophyte species and 2 controls; $\chi^2 = 658.47$, df $= 11$, $P < 0.001$). Post-rainfall simulation, the bryophyte colonies retained significantly more spores (90.7±7.4 %) than the controls (ceramic plate: 7.5±20.9 %; mudstone: 39.7±20.6 %; Tables 1, 2). Of the physical characteristics examined, average values of retained spores were strongly and positively correlated to dry weight per square centimeter ($R^2 = 0.62$, $P < 0.05$; Fig. 3A). We found no significant correlation between the percentage of retained spores and average colony height ($R^2 = 0.23$, $P > 0.05$; Fig. 3B) or leaf length ($R^2 = 0.18$, $P > 0.05$; Fig. 3C).

The average values of retained spores of *Thuidium cymbifolium* (Dozy & Molk.) Dozy & Molk. (81.4±9.8 %), *Jungermannia granulata* (Steph.) Amakawa (85.6±8.1 %), and *Hypnum oldhamii* (Mitt.) A. Jaeger & Sauerb. (86.7±9.3 %) were significantly lower than those of the other seven bryophytes (92.2±6.9–95.2±5.0 %; Tables 1, 2). These three species have low dry weight per square centimeter colony (13.9–14.8 mg/cm^2; Table 1).

DISCUSSION

Our study findings confirmed that bryophyte colonies retain more *Lycopodium* spores than the ceramic plates and mudstone blocks, regardless of bryophyte species. Therefore, in vertical environments such as surface of

TABLE 2. Significance (*P*) values Steel–Dwass nonparametric multiple comparison for spore-holding capacities of colonies of ten species of bryophytes and controls. Species and control acronyms heading the columns are listed in the same sequence as in the rows.

	CP	MB	*Bb*	*Cc*	*Ec*	*Ho*	*Jg*	*Jt*	*Km*	*Lb*	*Pa*	*Tc*
Ceramic Plates	1											
Mudstone Blocks	**<0.001**	1										
Brachythecium buchananii	**<0.001**	**<0.001**	1									
Ctenidium capillifolium	**<0.001**	**<0.001**	0.9974	1								
Entodon challengeri	**<0.001**	**<0.001**	0.9000	1	1							
Hypnum oldhamii	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	1						
Jungermannia granulata	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.9764	1					
Jungermannia truncata	**<0.001**	**<0.001**	1	0.9984	0.9259	**<0.001**	**<0.001**	1				
Kurzia makinoana	**<0.001**	**<0.001**	0.9819	1	1	**<0.001**	**<0.001**	0.9909	1			
Leucobryum bowringii	**<0.001**	**<0.001**	1	1	0.9997	**<0.001**	**<0.001**	1	1	1		
Plagiomnium acutum	**<0.001**	**<0.001**	0.0751	0.5942	0.7334	**<0.001**	**<0.001**	0.0745	0.7662	0.4208	1	
Thuidium cymbifolium	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.0034**	**0.0455**	**<0.001**	**< 0.001**	**< 0.001**	**< 0.001**	1

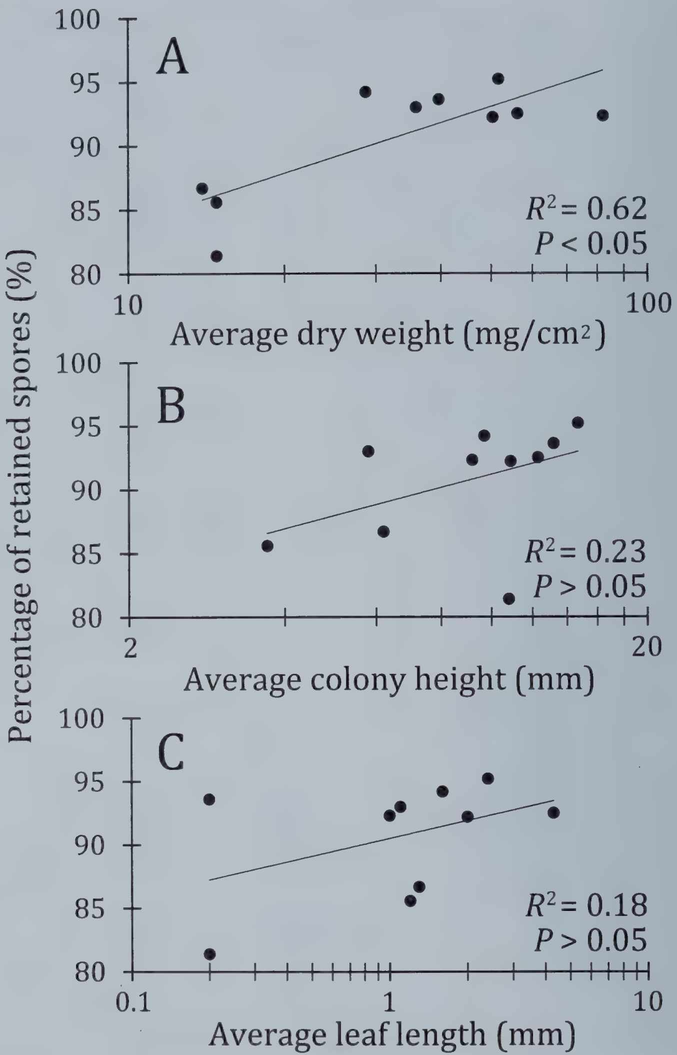

FIG. 3. The relationships between the average percentages of retained spores in the bryophyte mats and average values of colony height, leaf length, and dry weight per square centimeter of bryophyte colonies. Untransformed values of both retained spores and each bryophyte trait were plotted in the graphs. Pearson's correlation coefficients (R^2) and significance (P) were calculated using the arcsine-transformed average percentage values of retained spores and log-transformed values of each bryophyte trait.

rocks or cliffs, the spore-holding capacity of bryophyte colonies could positively affect pteridophyte establishment by acting as physical anchors for dispersing spores. Our results are consistent with those of previous studies suggesting that pteridophyte gametophytes frequently occur on bryophyte colonies in epiphytic or epipetric habitats (Mizuno *et al.*, 2012a, 2015).

All bryophyte species investigated in this study exhibited high average percentage values of retained spores (90.7±7.4 %). However, three species—*Thuidium cymbifolium*, *Jungermannia granulata*, and *Hypnum oldhamii*—had significantly lower average values of retained spores than those of most other bryophyte species. This difference was likely associated with species-specific variation in bryophyte physical characteristics that affects the surface on which spores can anchor.

In particular, we found that the average percentage values of retained spores were significantly correlated with dry weight per square centimeter, but not with colony height or leaf length. This is because the size of spores (28 μm) is considerably smaller than that of leaves (approximately 200–4300 μm). In addition, there were no lamellae on the surface of leaves in this study. Thus, the number of retained spores is likely to be a function of the total surface area of leaves and stems on which pteridophyte spores could adhere. Leaf thickness, consisting of single cell layer, and stem diameter were not considerably different across species. Thus, dry weight value of bryophyte colony per unit area is very likely a simple function of the number of stem-and-leaf units, which correlate closely with the surface area. Poor correlation of retained spores to colony height might be attributed to the differences in the density of stem-and-leaf units per volume, which is responsible for the variation in surface area per volume across species. Leaf length might not be correlated with the total surface area because of the interspecific variation of leaf width and stem length. We investigated only three characteristics of bryophytes without lamellae, such as colony height, leaf length, and dry weight. However, spore retention within bryophyte colonies of species other than those investigated in this study might also depend on the structures that increase the surface area, such as leaf lamellae as well as folding of leaves and branching of stems.

In the present study, we showed that bryophyte colonies help pteridophyte spores adhere to epiphytic and epipetric habitats. However, we found that the amount of retained spores differed by approximately 10% across bryophyte species. This low level of variation could possibly be explained by the fact that we only simulated one type of rainfall, i.e., moderate rainfall (20 mm/h, 10 min). Thus, inter-specific differences in spore retention during rainfall might have been underestimated. Future studies are needed to identify bryophyte colonies that are stronger or have more sustenance to rainfall. A more fine-tuned manipulation of rainfall strength might clarify any differences in spore-holding capacity because of precipitation.

Finally, variation in pteridophyte establishment on vertical environments might be attributed to factors other than spore-holding capacity of bryophyte colonies. For example, perispore ornamentations might affect the ability of spore adherence in bryophyte colonies. Comparing the ability of spore adherence depending on perispore ornamentation is important for clarifying suitable perispore structures for spore retention in a vertical environment. Shading effects of bryophytes also might adversely affect gametophyte growth. Gametophytes of pteridophyte are commonly found on tree trunks or rock cliffs in bryophyte colonies consisting of species that have low colony height

(Mizuno *et al.*, 2012a, 2015). Although dense bryophyte colonies could have high spore-holding capacity, growth of pteridophyte gametophyte is susceptible to competition from the bryophytes (Watkins *et al.* 2007). Thus, bryophyte colonies that have high spore-holding capacity are not always suitable for the establishment of epiphytic and epipetric pteridophytes. In addition, bryophyte competition in the form of allelopathy might be a critical factor that affects pteridophyte establishment. Bryophyte allelopathy is well established (e.g., Michel *et al.*, 2011) and, depending on the interacting species, such competition could either promote or prevent spore germination of pteridophyte. In conclusion, further studies are required to completely understand the complex factors that influence pteridophyte establishment in epiphytic and epipetric habitats.

Acknowledgments

This study was financially supported by a Sasakawa Scientific Research Grant from The Japan Science Society. We are deeply indebted to Dr. Tatsuwo Furuki of the Natural History Museum and Institute, Chiba, for identifying bryophytes.

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American Fern Journal 106(3):171–174 (2016)
Published on 19 October 2016

Nomenclatural Note on the Pteridophyte Flora of the Guianas

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ABSTRACT.—The study of the pteridophytes of the Guiana Shield and the writing of the Flora of the Guianas led to the discovery that, taxonomically, a few combinations were not yet established for certain taxa. Accordingly, new combinations are proposed for two subspecies of Hymenophyllaceae (**Didymoglossum punctatum** subsp. **labiatum** and **D. punctatum** subsp. **sphenoides**), two varieties of Pteridaceae (**Jamesonia paucifolia** var. **neblinae** and **J. paucifolia** var. **steyermarkii**) and one species of Thelypteridaceae (**Amauropelta demerarana**).

KEYWORDS.—Hymenophyllaceae, Pteridaceae, Thelypteridaceae, *Didymoglossum, Jamesonia, Amauropelta*, taxonomy, Neotropics

RÉSUMÉ.—L'étude des ptéridophytes du Plateau des Guyanes et la rédaction de la Flore des Guyanes ont montré que, au niveau taxonomique, des combinaisons n'avaient pas encore été établies pour certains taxons. De nouvelles combinaisons sont donc proposées pour deux sous-espèces d'Hymenophyllaceae (**Didymoglossum punctatum** subsp. **labiatum** et **D. punctatum** subsp. **sphenoides**), deux variétés de Pteridaceae (**Jamesonia paucifolia** var. **neblinae** et **J. paucifolia** var. **steyermarkii**) et une espèce de Thelypteridaceae (**Amauropelta demerarana**).

MOTS-CLÉS.—Hymenophyllaceae, Pteridaceae, Thelypteridaceae, *Didymoglossum, Jamesonia, Amauropelta*, taxonomie, Néotropiques

During our work on the pteridophytes of the Guianas, our attention was focused on the typification of taxa and we have established a list of all taxa possessing type specimens (at least a syntype) collected from the Guianas (Cremers & Boudrie, 2007).

The progress of our work and the continuous follow-up of new publications related to Neotropical ferns, especially those from the Guianas, led us to the conclusion that certain taxonomic combinations were needed. These missing combinations concern taxa from Hymenophyllaceae (*Didymoglossum*), Pteridaceae (*Jamesonia*) and Thelypteridaceae (*Amauropelta*).

NEW COMBINATIONS

Hymenophyllaceae.—This family has been treated in the third fascicle of the *Flora of the Guianas* (Lellinger, 1994) in which the following taxon is presented. Here we present the barcode number of the holotype:

Trichomanes (*Didymoglossum*) *punctatum* Poir., Encycl. 8: 64. 1808. TYPE: MARTINIQUE. coll. not stated (HOLOTYPE: P [barcode P00564896!]).

This binomial was used by Pacheco (1995) and Sánchez (2000), but Ebihara *et al.* (2006), in their revision of Hymenophyllaceae, used the combination *Didymoglossum* (subg. *Didymoglossum*) *punctatum* (Poir.) Desv. Recently the latter binomial was used by Christenhusz (2009) and Windisch (2010, 2014). When making the combination in *Didymoglossum,* however, Ebihara *et al.* (2006) did not mention or establish the combinations for the subspecies of *D. punctatum*. For the Guianas and nearby countries, there are two taxa concerned:

a) Subsp. *labiatum*

Didymoglossum punctatum (Poir.) Desv. subsp. ***labiatum*** (Jenman) Boudrie & Cremers, ***comb. nov.*** *Trichomanes labiatum* Jenman, Gard. Chron. ser. 2, 24: 7. 1885. *Trichomanes punctatum* Poir. subsp. *labiatum* (Jenman) Wess. Boer, Acta Bot. Neerl. 11: 302. 1962. TYPE: GUYANA. Essequibo River from Bartica Grove, Aug 1884, *G. S. Jenman 2109* (HOLOTYPE: K [barcode 000589509!]).

The distribution of this subspecies extends from Central America (Nicaragua, Costa Rica) to South America (Colombia, Trinidad, Tobago, Venezuela, the three Guianas, Peru, Bolivia, Brazil).

b) Subsp. *sphenoides*

As the existence of this subspecies was accepted by Proctor (1985), Lellinger (1989), Proctor (1989), Tryon & Stolze (1989), Pacheco (1995), Sánchez (2000), and Sundue (2010), and that *Trichomanes sphenoides* Kunze is considered by Ebihara *et al.* (2006) as belonging to *Didymoglossum*, we make the following combination:

Didymoglossum punctatum (Poir.) Desv. subsp. ***sphenoides*** (Kunze) Boudrie & Cremers, ***comb. nov.*** *Trichomanes sphenoides* Kunze, Linnaea 9: 102. 1834. *Trichomanes punctatum* Poir. subsp. *sphenoides* (Kunze) Wess. Boer, Acta Bot. Neerl. 11: 301. 1962. TYPE: PERU. Huánuco: from Cuchero ad Pampayaco, 1829, *E. F. Poeppig s.n.* [Diar. 1167] (LECTOTYPE, designated by Wessels Boer, 1962: W [accession no. W0052726!]; ISOLECTOTYPES: B [barcode 20-0083918!], MO [barcode 255410]; possible original material P [barcodes P00624533!, P01303683!]).

This taxon is present in the Greater Antilles, Central America (from Guatemala and Belize to Panama), and northwestern South America (Colombia, Ecuador, Venezuela, Peru, Bolivia).

Pteridaceae.—Jamesonia paucifolia (A. C. Sm.) Christenh., Phytotaxa 19: 21. 2011. *Psilogramme paucifolia* A. C. Sm., Bull. Torrey Bot. Club 58: 305. 1931. *Eriosorus paucifolius* (A. C. Sm.) Vareschi, *in* Lasser, Fl. Venez. 1: 641. 1969. TYPE: VENEZUELA. Bolívar: 50 ft. below summit of peak no. 7, summit of Mt.

Duida, 7050 ft. [2150 m], Aug 1928–Apr 1929, *G. H. H. Tate 677* (HOLOTYPE: NY [barcode 144517!]; ISOTYPES: K [barcode 000633134], US [barcode 00142133]).

The varieties mentioned by Tryon (1970) in her monograph of *Eriosorus* were not cited by Christenhusz *et al.* (2011). *Jamesonia paucifolia* is restricted to Venezuela (States of Amazonas and Bolívar). Both vars. *neblinae* and *steyermarkii* are limited to the Roraima massif, which is at the junction between Venezuela, Brazil, and Guyana. Therefore, it probably also occurs in Guyana. For the moment, Funk *et al.* (2007) and Hokche *et al.* (2008) consider these varieties as morphotypes of the species. Although we have not been able to verify the distinction of these varieties, we propose the new combinations below:

Jamesonia paucifolia (A. C. Sm.) Christenh. var. ***neblinae*** (A. F. Tryon) Boudrie & Cremers, ***comb. nov.*** *Eriosorus paucifolius* (A. C. Sm.) Vareschi var. *neblinae* A. F. Tryon, Contr. Gray Herb. 200: 103, fig. 13. 1970. TYPE: VENEZUELA. Amazonas: Cerro de la Neblina, Río Yatua, 1400–1700 m, 24 Jan 1954, *B. Maguire, J. J. Wurdack & G. S. Bunting 37381* (HOLOTYPE: US [barcode 00142134]; ISOTYPES: COL [barcode 000006233], NY [barcode 02597504!], UC [barcode 1484782!], VEN [not seen]).

Jamesonia paucifolia (A. C. Sm.) Christenh. var. ***steyermarkii*** (A. F. Tryon) Boudrie & Cremers, ***comb. nov.*** *Eriosorus paucifolius* (A. C. Sm.) Vareschi var. *steyermarkii* A. F. Tryon, Contr. Gray Herb. 200: 103, fig. 13. 1970. TYPE: VENEZUELA. Bolívar: Ptari-tepui, along base of south-facing high sandstone bluffs, 2410 m, 6 Nov 1944, *J. A. Steyermark 59913* (HOLOTYPE: US [barcode 00588551!]; ISOTYPES: NY [barcode 00169714], S [accession no. S-R-5571], VEN [barcode 82247]).

Thelypteridaceae.—The publication of the recent revision of Neotropical Thelypteridaceae (Salino *et al.*, 2015) led us to update our list of taxa for the Guianas. The conclusions of a discussion with Alan R. Smith about the new names of previous *Thelypteris* were that the combination of *Thelypteris demerarana* in *Amauropelta* (in which genus it is related; cf. Smith, 1993) did not exist, despite the presence of the combination *Amauropelta demerarana* Baker mentioned on the K web site, at the name of *Polypodium demeraranum,* which is an obvious error (A. R. Smith, pers. comm.). Therefore, to fill this gap, we propose the following combination:

Amauropelta demerarana (Baker) Boudrie & Cremers, ***comb. nov.*** *Polypodium demeraranum* Baker, Timehri 5: 214. 1886. *Dryopteris demerarana* (Baker) C. Chr., Index Filic. 261. 1905. *Lastrea demerarana* (Baker) Vareschi, Flora Venez. 1: 430. 1969. *Thelypteris demerarana* (Baker) C. F. Reed, Phytologia 17(4): 271. 1968. TYPE: VENEZUELA. Roraima, found on the upper slopes of the mountain, Dec. 1884, *E. F. im Thurn 356* (LECTOTYPE: K [barcode 000633499!, designated by Smith, Flora of the Guianas, Fasc. 6 : 89. 1993]; ISOLECTOTYPES: K [barcode 000633500!, second-step lectotypification designated here], BM (not seen), US [barcode 00066573!]). SYNTYPE: GUYANA, Roraima, British Guiana, s.d. [date not visible, label unfortunately cut, but prob. 1863], *C. F. Appun* 1138, K [barcodes 000501721!, 000501722!].

ACKNOWLEDGMENTS

We are very grateful to Warren D. Hauk, Fernando Matos, Robbin C. Moran and Alan R. Smith for their valuable remarks and considerable improvement of the text, as well as to Anna Haigh, Robbin C. Moran, Laura Pearce and Alan R. Smith for their help in providing us images of type specimens from K, NY and UC.

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American Fern Journal 106(3):175–190 (2016)

Published on 19 October 2016

Three new scaly tree fern species (*Cyathea*-Cyatheaceae) from the Amotape-Huancabamba Zone and their biogeographic context

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Abstract.—Three species of the genus *Cyathea* from the sandstone areas of the Amotape-Huancabamba zone are described as new to science: *Cyathea abrapatriciana* and *C. oreopteroides* from northern Peru and *C. chimaera* from southern Ecuador. They are illustrated and discussed regarding their biogeography and putative relationships.

Key Words.—Andes, Ecuador, *Hymenophyllopis*, Neotropics, Peru, sandstone, South America

The Amotape-Huancabamba zone, straddling the border between Ecuador and Peru, is a relatively low-lying stretch of the Andes known to harbor one of the richest floras of any similar-sized area anywhere in the World (Schulenberg and Awbrey 1997; Ayers 1999; Weigend 2002; Neill, 2005). The region, which extends from the Pacific coast of South America to the Amazon headwaters at middle basin of the Marañón river, is characterized by a large east to west humidity gradient, nearly aseasonal climate, a diverse geology including metamorphic rocks as well as limestone and sandstone, and a highly rugged topography of ridges and plateaus isolated by deep river valleys. This diversity of climate and geology, in combination with natural habitat fragmentation are often proposed as explanations for the high biological diversity of this area (Knapp 2002; Weigend 2002, 2004).

A geologic peculiarity of this region is the widespread presence of cretaceous sandstone derived from the weathering of the much older Guayana shield sandstones and granites (Campbell, 1974; Gibbs and Barron, 1993). The sandstone outcrops, usually occurring as isolated ridges and plateaus, support rich botanical communities that stand in sharp contrast to surrounding Andean montane forests and contain disjunct representatives of taxa known otherwise exclusively from the Guayana shield (Berry and Riina, 2005), as well as many local endemics (Neill 2005).

The tree ferns (Cyatheaceae) are one group of plants that shows a striking pattern of narrow endemism, with several species restricted to one or a few sandstone mountain tops, and some known only from their type localities (Domin, 1929; Moran, 1995; León and Moran, 1996; Smith, 2006; Lehnert, 2006; Lehnert, 2011a; Lehnert, 2011b). Here we describe three new tree fern species from sandstone areas of the Amotape-Huancabamba zone, providing additional evidence of the floristic uniqueness and endemism of the region.

MATERIALS AND METHODS

Field trips to Peru were done in November 2010 and July 2014, and to Ecuador in July 2014. Comparative material was studied at the herbaria BONN, STU, and QCA, and online resources were used for checking type specimens (plants.jstor.org) and historic literature (www.botanicus.org; www.biodiversitylibrary.org). Terminology follows Lellinger (2002) and Lehnert (2011b).

TAXONOMIC TREATMENT

Cyathea abrapatriciana Lehnert & A.Tejedor, ***sp. nov.*** Type:–PERU. San Martín: Abra Patricia, between Pedro Ruíz and Rioja, 05°41′S, 77°48′W, 2000–2100 m, 5 Nov 2010, *Lehnert 2845* (holotype: USM [2 sheets]; isotypes: BONN [3 sheets], Z). **Figs. 1, 2A.**

A new species of *Cyathea* with hemitelioid indusia and bipinnate-pinnatifid to tripinnate laminae. It stands out among its neotropical congeners by having larger scales only on the croziers, matching in this respect only the sphaeropteroid indusiate *C. planadae*, which differs in having strongly aculeate petioles, not inermous as in *C. abrapatriciana*.

Tree fern, trunk to 2.7 m tall, erect, without old petiole bases, to 7 cm diam., scales only on the exposed apex between the petioles and on the croziers, epidermis medium to dark brown, with a fine cover of easily abraded scurf, inconspicuous, frond scars long elliptic, to 6 × 3 cm, remote form each other, each subtended by an arc of few, relatively large orange-brown elliptic lenticels to 40 × 12 mm; adventitious buds absent. Fronds to 240 cm long, loosely spirally arranged around apex (no fascicle), strongly arching, the tips drooping. Petioles to 90 cm long, inermous, green or with some dark brown areas when older, with similar scurf as trunk, soon worn off, with an interrupted line of elongate pneumathodes on each side, to 25 × 2 mm, first white, eventually becoming brown; petioles scales only present in croziers, soon caduceous in fully expanded fronds, ovate to ovate-lanceolate, to 11.0 × 3.5 mm, dull medium brown, with paler margins grayish white (in dried material, appearing darker in fresh material), not sharply contrasted, margins rather firm, with strongly exerted cell rows, ending in most parts in brown marginal teeth. Laminae to 150 × 120 cm, ovate-lanceolate, bipinnate-pinnatisect to tripinnate, dark green adaxially (dark olive when dried), paler grayish green abaxially, with 14–16 pinna pairs, often opposite or nearly so, basal ones strongly reflexed, not or scarcely shorter than longest central pinnae. Frond axes inermous, green to yellow, abaxially sometimes also partially atropurpureous or dark brown; costae basally with a small elliptic pneumathode, ca. 3 × 1 mm, greyish-brown, weakly raised, with a flat darker spot below (gland), costae narrowly green-alate between the pinnules in distal

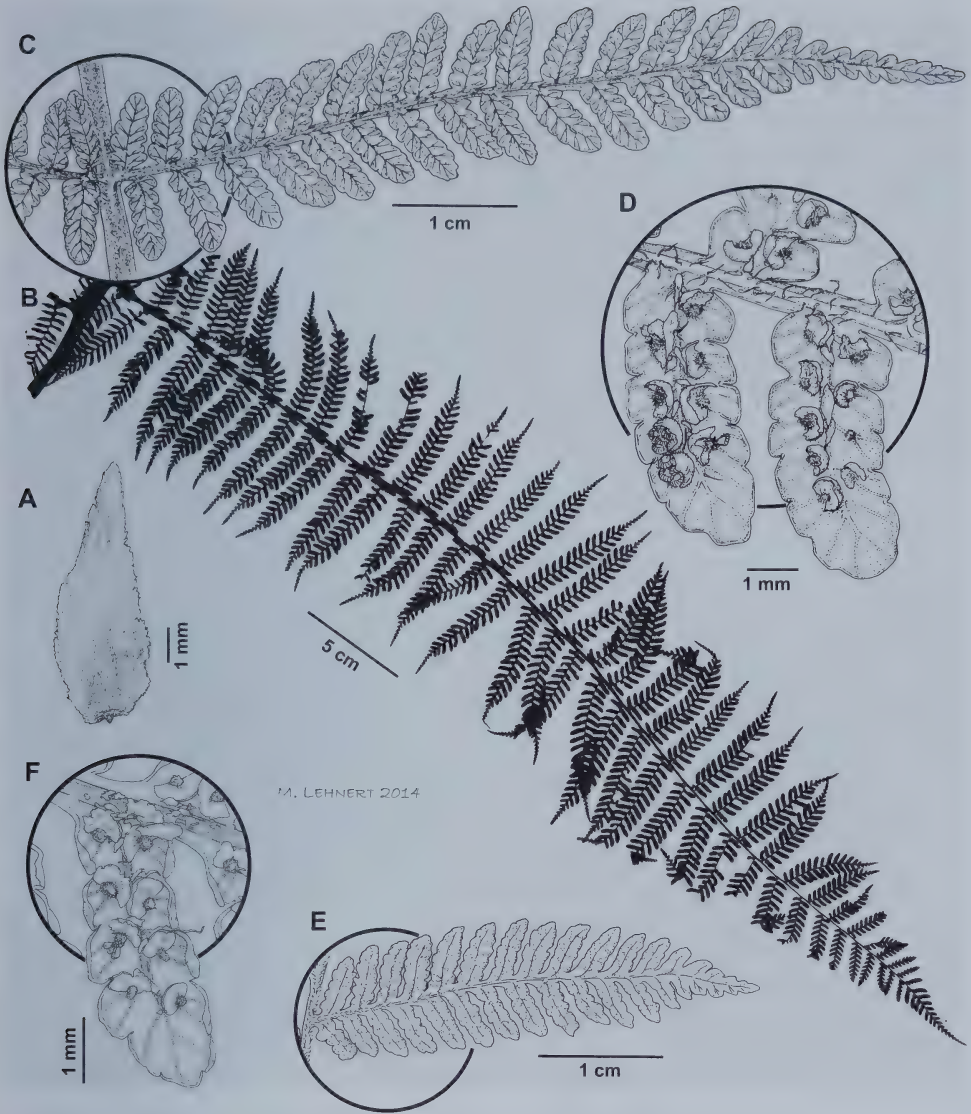

FIG. 1. A–E. *Cyathea abrapatriciana*. A. Petiole scale. B. Medial pinna. C. Pinnule, adaxially. D. Fertile segments abaxial surface, showing large, almost semicircular indusial. (From isotype, *Lehnert 2845*, BONN). E–F. *Cyathea cicatricosa*: E. Pinnule, adaxial surface. F. Fertile segment, abdaxially. (From the holotype, *Green 1778*, K.)

half. Longest pinnae 52–60 ×12 cm, linear-lnaceolate, sessile, towards the lamina base also stalked to 2.5 cm. Largest pinnules to 60 × 11 mm, linear-lanceolate with attenuate tips, sessile to subsessile, with weakly cordate bases, fully pinnate in lower half, basal segments free; segments to 6 × 2 mm, the margins coarsely crenate, in larger ones also lobed almost to the midvein, veins flat to weakly raised abaxially, flat to weakly immersed adaxially, fertile veins

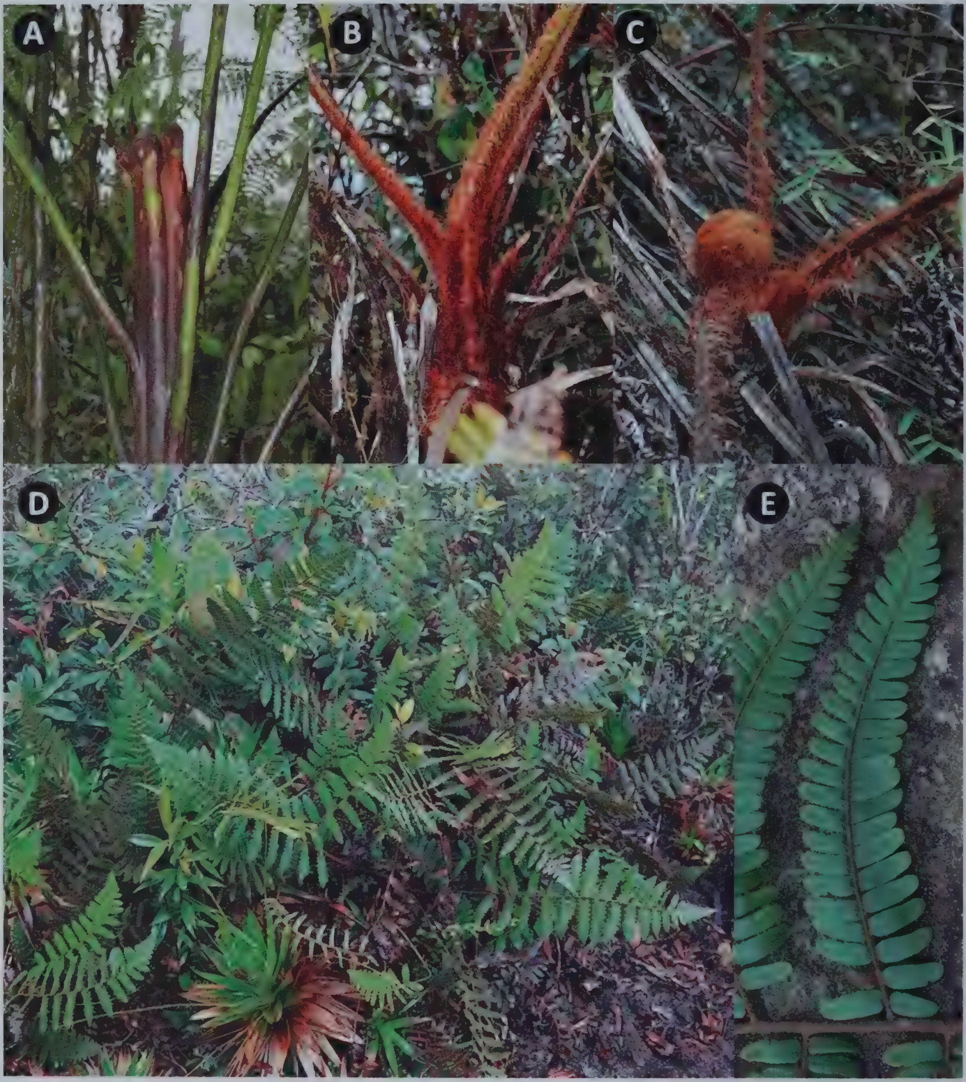

FIG. 2. Field characters of the newly described species of *Cyathea* and their most similar congeners. A. *Cyathea abrapatriciana*, trunk showing brown epidermis, orange lenticels and restriction of scales to croziers. (Abra Patricia, Prov. Amazonas, Peru; © M. Lehnert) B. *Cyathea chimaera*, trunk apex and petioles bearing fine orange-brown scales that at first glance appear like a hairy indument. (Las Orquideas, Prov. Zamora-Chinchipe, Ecuador; © M. Lehnert) C. *Cyathea aterrima*, showing same features as in B. (Cerro Tayu, Prov. Amazonas, Peru; © M. Lehnert). D. *Cyathea oreopteroides*, group of plants showing erect position of the fronds. (Abra Patricia, Prov. Amazonas, Peru; © M. Lehnert) E. *Cyathea thelypteroides*, large pinna, showing pinnate dissection in lower part, a feature previously unknown for the species (Cerro Tayu, Prov. Amazonas, Peru; © M. Lehnert).

forked. Sori proximal, to ca. 0.8 mm diam., each with ca. 10–20 sporangia, deeply castaneous in fresh material, indusia hemitelioid, reaching 1/4–1/3 around the receptacles, ascending, with erose margins, papery, pale brown when dry, translucent and inconspicuous when wet, receptacles globose, ca. 0.1–0.2 mm diam., paraphyses few, straight, shorter than sporangia, < 0.2 mm long, whitish. Spores pale yellow. Hairs and scales: Frond axes adaxially with pale brown to white, sometimes translucent multicellular hairs, antrorsely curved ones 1.0–2.0 mm long, and longer spreading ones, to 3.5 mm long, quite dense except for central and lower rhachis, abaxially with hairs sparser and more spreading but similarly distributed, veins lacking hairs or midveins with few scattered hairs on both sides; pale brown bullate squamules abaxially frequent on costules and abundant on midveins, on the former usually narrower than on the latter.

ETYMOLOGY.—The specific epithet refers to the type locality.

DISTRIBUTION AND HABITAT.—*Cyathea abrapatriciana* is currently known from only one locality in the Andes, occurring on humus rich soils over white sandstone under perhumid conditions, at 2000–2100 m in the understory of dense mountain rainforest.

We first mistook *Cyathea abrapatriciana* in the field for *C. multigsegmenta* R.M.Tryon from central and southern Peru, which also has slender trunks and highly dissected fronds. *Cyathea abrapatriciana* differs, however, by hemitelioid indusia (vs. complete sphaeropteroid in *C. multisegmenta*), and darker petiole scales (brown with paler brown margins, appearing brown *en masse* vs. broad whitish margins and pale brown center, appearing whitish *en masse*) that are present only on croziers (vs. ± persistent cover from the trunk to most parts of the petioles). *Cyathea abrapatriciana* also resembles *C. moranii* Lehnert, a species of southern Ecuador (see Lehnert 2006). However, *C. moranii* has sphaeropteroid indusia (vs. hemitelioid in *C. abrapatricana*) and largely lacks hairs abaxially; the petioles are usually dark brown in mature fronds (vs. long time green), the scales on the petioles and trunk apices are larger (to 22.0 × 8.0 mm vs. to 11.0 × 3.5 mm), longer persistent on the trunk apex, more bicolorous with wider, paler margins that dominate the color of the scale cover (vs. scales *en masse* rather dark). *Cyathea holdridgeana* from Central America can be separated from *C. abrapatriciana* based on the same differences, because it matches *C. moranii* in all characters almost perfectly except for the indusia, which are hemitelioid like in *C. abrapatriciana*, albeit a bit smaller. *Cyathea abrapatriciana* further resembles *C. platylepis* (Hook.) Domin from the Guayana Highlands, which also grows on sandstones and agrees in the hairiness and the relatively large hemitelioid indusia, but which differs in having fewer bullate squamules (sparse abaxially on costules in *C. platylepis* vs. abundant in *C. abrapatriciana*) and more serrate segment margins (lobes acute vs. lobes rounded). Also, *C. platylepis* has persistent scales on the petiole and the trunks (pers. observation), whereas *C. abrapatriciana* has the scales caduceus and usually only present on the croziers.

Cyathea abrapatriciana is remarkable because from its appearance in field, it is more similar to *C. decurrens* Copel. and allies from the western Pacific area rather than to any Neotropical one (Lehnert, 2011b). It agrees well with these species regarding appearance, the dissection of the lamina, the morphology of the indument, and the hemitelioid indusia. The size and shape of the indusia are reminiscent of the New Caledonian species *C. cicatricosa* Holttum (Fig. 1F). *Cyathea abrapatriciana* has a unique mosaic of characteristics so that it cannot be confused with any of these Paleotropical species, especially the presence of foliar glands in the axils of rhachises and costae abaxially, which the Paleotropical species lack (White and Turner, 2012).

At the type locality, *Cyathea abrapatriciana* was found in large numbers as juvenile, trunkless plants along the trail but only at one spot as a fertile plant in the understory. That plant has shown a considerable growth rate, since it added ca. 50 cm or almost 25% in trunk height within six months (A. Tejedor, pers. obs.). This indicates the presence of a typical life strategy as a pioneer species that establishes quickly on raw soils in clearings, and then persists with few individuals as the forest recovers, only to vanish when the canopy becomes too dense. A similar strategy has been observed for several species of the Paleotropical *C. decurrens* and the neotropical *C. vilhelmii* group (Lehnert, 2011b, pers. obs.).

Cyathea chimaera Lehnert & A.Tejedor, ***sp. nov.*** TYPE.—ECUADOR. Zamora-Chinchipe: Hill behind Yancuam lodge, near Las Orquideas, –4.259068, –78.694725, 1800 m, 30 Jun 2014, *M. Lehnert et al. 2791* (holotype: HUTPL; isotypes: BONN, Z). **Figs. 2B, 3.**

A new species of *Cyathea* with bipinnate-pinnatifid fronds and complete sphaeropteroid indusia that most closely resembles *C. dudleyi* R.M.Tryon in stature and frond dissection but differs in having a dense indument of hairs and much narrower petiole scales that appear almost like hairs. Other species of *Cyathea* with similarly narrow scales and dense hair lack indusia.

Tree fern, trunk to 1.5 m tall, erect to decumbent, with old petiole bases, to 6 cm diam., hair-like scales like those on the petioles between the petioles and on the croziers, epidermis dark brown, inconspicuous, frond scars usually not visible; adventitious buds absent. Fronds to 140 cm long, arranged in a fascicle around trunk apex, ± patent on ascending petioles. Petioles to 60 cm long, inermous, green or dark brown when older, densely hairy with multicellular hairs to 2 mm long, pneumathodes narrow dark-green slits ca. 10–12 × 0.8–1.0 mm, hidden between scales; petioles scales dense near the petiole bases, thinning towards rhachis, long-lasting, appearing hair-like, linear-lanceolate, to 25.0 × 1.5–2.0 mm, with tortuous tips, concolorous fuscous brown or with paler margins, not sharply contrasted, margins rather soft, without teeth or ciliae. Laminae to 80 × 55 cm, ovate, bipinnate-pinnatifid to -pinnatisect, with gradually reduced apex, shiny medium green adaxially (dark olive when dried), paler matte grayish green abaxially, with ca. 12 pinna pairs, ± alternate, basal ones strongly reflexed, not much shorter than longest central pinnae. Frond

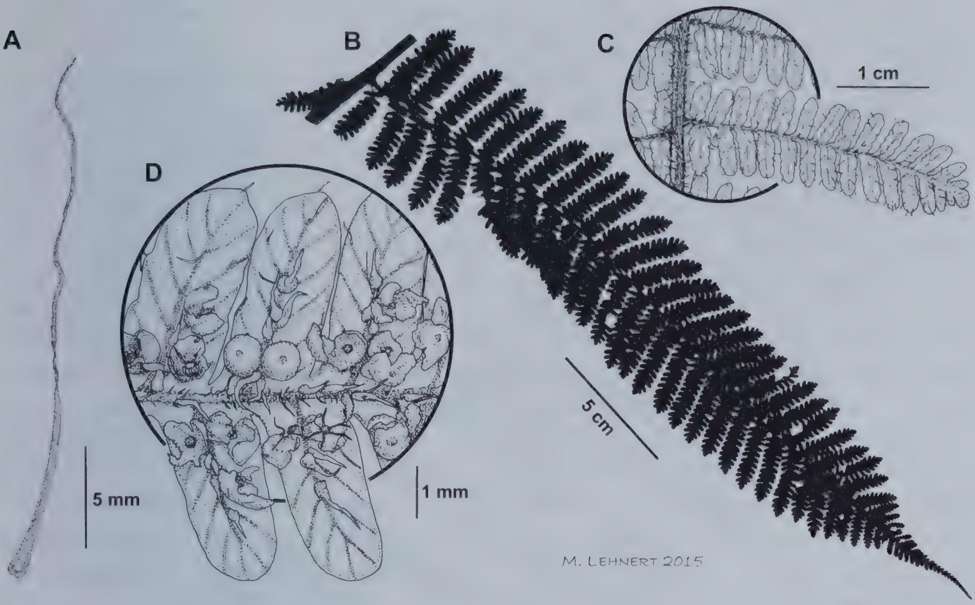

FIG. 3. *Cyathea chimaera.* A. Petiole scale. B. Medial pinna. C. Fertile pinnules, adaxially. D. Fertile segments, abaxially, showing sphaeropteroid indusial with umbo. (From the isotype, *M. Lehnert 2791*, BONN.)

axes inermous, green to yellow; costae basally with 1-2 elliptic pneumathodes to 2 × 1 mm, grayish, also with green spot lacking indument, weakly raised in fresh material (foliar gland); costae green alate between the pinnules in distal half. Longest pinnae 18 × 5 cm, linear, sessile. Largest pinnules to 26 × 12 mm, elongate with obtuse tips, sessile, with weakly cordate bases, fully pinnate in lower half, basal segments free; segments to 7 × 3 mm, weakly ascending, oblong with round tips, the margins weakly crenate to subentire, veins flat to weakly raised abaxially, flat adaxially, fertile veins mostly simple, rarely forked. Sori medial, to ca. 0.8 mm diam., each with ca. 20–30 sporangia, matte brown in fresh material, indusia sphaeropteroid with apical umbo, receptacles globose, ca. 0.2 mm diam., paraphyses few, straight, shorter than sporangia, < 0.2 mm long, whitish. Spores bright yellow. Hairs and scales: Frond axes and veins on both sides with white, sometimes translucent multicellular hairs to 2 mm long, quite dense on larger axes, sparser on veins, costules and midveins abaxially also with orange-brown bullate squamules 0.5–1.0 × 0.5 mm.

ETYMOLOGY.—The specific epithet refers to resemblance of this species to a grafting of several other species.

DISTRIBUTION AND HABITAT.—*Cyathea chimaera* is known only from one locality in the Ecuadorean Andes, growing on humus rich soils over brown sandstone under perhumid conditions, in open ridge-top vegetation at 1800 m.

Cyathea chimaera has a stature and laminar dissection similar to *C. dudleyi,* a widespread species regularly found in southern Ecuador. *Cyathea dudleyi* resembles *C. chimaera* by sphaeropteroid indusia. Both species can be separated at first glance because *C. chimaera* is densely hairy on the blades (vs. blades glabrous to glabrescent in *C. dudleyi*) and has narrow hair-like petiole scales. *Cyathea obnoxia* Lehnert is another hairy, sphaeropteroid indusiate species that occurs in the area, but it differs by being larger, the pinnule shape (tips ± acute to short tapering in *C. obnoxia* vs. obtuse to round in *C. chimaera*), and most importantly the size and shape of the petiole scales (22 × 3–5 mm, rather broadly lanceolate vs. 25.0 × 1.5–2.0 mm, linear lanceolate). The petiole scales of *C. chimaera* are almost indistinguishable from those of *C. rufescens* (Mett.) Domin from northern Peru (see Lehnert, 2011b), which differs by much larger pinnules, hemitelioid indusia, firmer laminar texture, and no hairs on the leaves. *Cyathea aterrima* (Hook.) Domin is a variable and widespread but elusive species that also has thin petiole scales that resemble hairs at first glance (Fig. 2C). It has the same soft laminar texture of *C. chimaera* and is found under similar edaphic and microclimatic conditions, although usually at lower elevations. It can be easily distinguished by the absence of indusia and the presence of long paraphyses.

The discovery of *Cyathea chimaera* was surprising because the Ecuadorian province of Zamora-Chinchipe is one of the botanically best-studied areas in the Andes (Beck *et al.*, 2008). The type locality had been visited before by Henk van der Werff (MO), who has a talent for finding rare fern species. During his visit he discovered *C. brucei* Lehnert, which grows on shaded sandstone escarpments below the ridge where *C. chimaera* is found. He also found other rare ferns such as *C. latevagans* (Baker) Domin, *C. palaciosii* R.C. Moran, and *Pterozonium reniforme* (Mart.) Fée.

Cyathea oreopteroides Lehnert & A.Tejedor, ***sp. nov.*** TYPE.—PERU. San Martín: Abra Patricia, between Pedro Ruíz and Rioja, 05°41′S, 77°48′W, 2000–2100 m, 5 Nov 2010, *M. Lehnert 2846* (holotype: BONN; isotype: USM). **Figs. 2D, 4.**

A small exindusiate *Cyathea* with pinnate-pinnatifid fronds with rather short (± 1 mm long) hairs abaxially on frond axes and veins, in this regard being intermediate between the similar *C. thelypteroides* A.R.Sm., which largely lacks hairs, and *C. phegopteroides* (Hook.) Domin, which has hairs to 2 mm long especially on the rhachis.

Terrestrial or small tree fern, trunk to 0.6 m tall, ascending, with persisting old petiole bases, to 3 cm diam., apex hidden between the petioles and croziers, epidermis medium to dark brown, lenticels not visible, adventitious buds present, forming small clumps of plants. Fronds to 70 cm long, tightly spirally arranged around apex (forming an elongated fascicle), strongly ascending, the tips pointing up or sideways. Petioles to 17 cm long, inermous, dull brown, pneumathodes not observed, with pulverulent scurf consisting of discrete crested orange-brown to brown squamules 0.2–0.5 long; petioles scales reaching up to the rhachis, persisting in fully expanded fronds (but

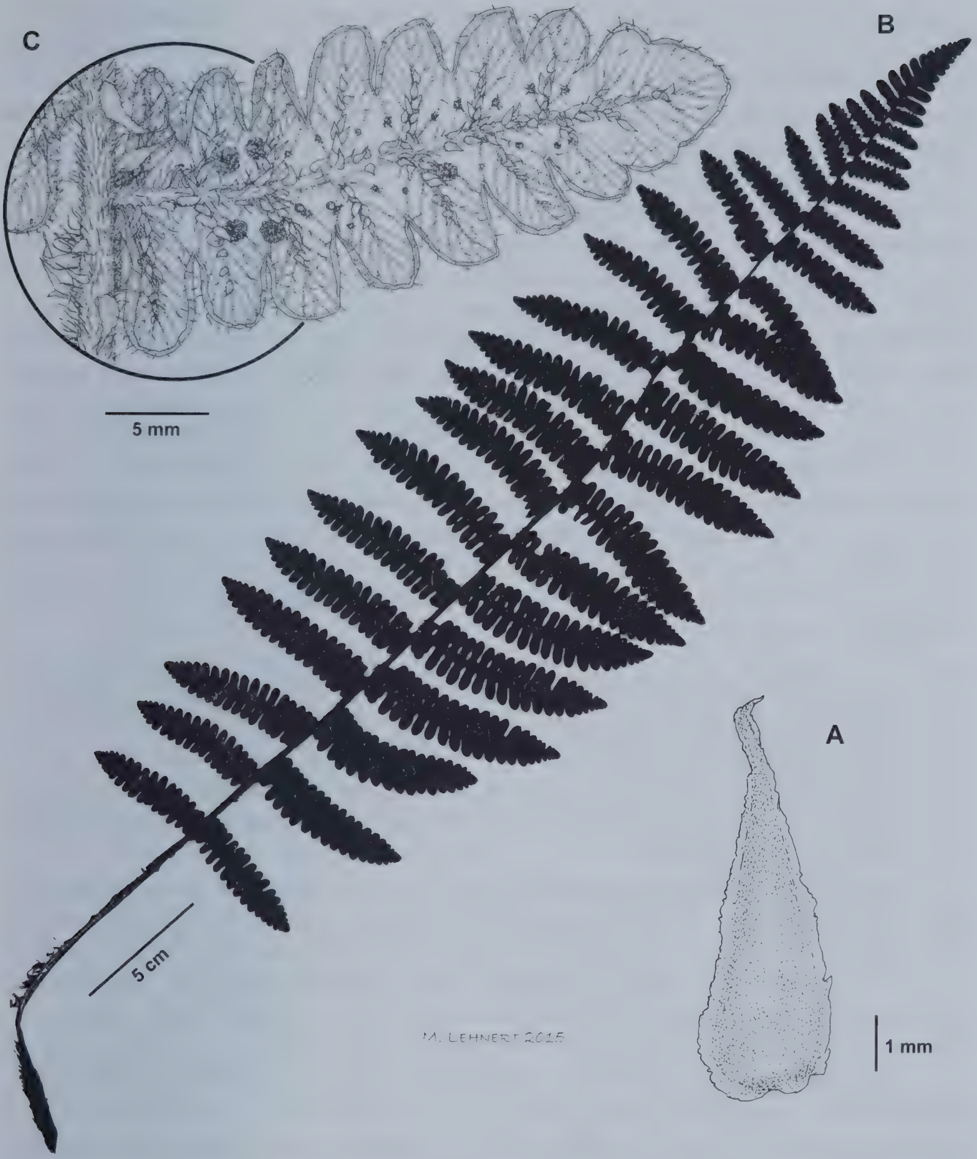

FIG. 4. *Cyathea oreopteroides.* A. Petiole scale. B. Whole frond, silhouette. C. Fertile pinna from apical section, abaxially. (From the isotype, *Lehnert 2846,* BONN.)

caduceous in dried specimens), narrowly lanceolate, to 17.0 × 2.5 mm, brown to reddish brown with slightly paler margins, not sharply contrasted, margins rather firm, without darker marginal teeth. Laminae to 55.0 × 18.5 cm, subcoriaceous, appearing slightly succulent, linear-lanceolate, pinnate-pinnatifid to -pinnatisect, dark grayish green adaxially (brown when dried), the same color or slightly paler abaxially, with 12–16(–20) pinna pairs, usually alternate except for basal ones, these patent or very weakly reflexed, at least 3/4 the length of the longest central pinnae. Frond axes inermous, dark orange-brown to blackish brown, abaxially sometimes also partially atropurpureous or black; costae basally with round pneumathode to 1 mm diam., in fresh material also with pale green elliptic spot to 2 × 1.5 mm (foliar gland). Longest pinnae linear-lanceolate, 9.6 × 2.2 cm, sessile, margins lobed 2/3 to almost to the costae; segments to 13 × 6 mm, oblong with tips obtuse to rounded, the margins subentire, veins flat to weakly raised abaxially, flat to weakly immersed adaxially, fertile veins forked. Sori medial, to ca. 0.8 mm diam., each with ca. 20 sporangia, pale orange-bown, exindusiate, receptacles globose, ca. 0.1–0.2 mm diam., paraphyses few, straight, shorter than sporangia, < 0.2 mm long, brown. Spores not examined. Hairs and scales: Frond axes on both sides with minute reddish brown scales ca. 0.5 mm diam. and short appressed hairs to 0.5 mm long, adaxially also with many appressed linear lanceolate scales to 2.5 × 0.5 mm, costae and midveins/costulae on both sides with brown to reddish multicellular hairs, erect to spreading, 0.5–1.5 mm long, hairs also evenly distributed on and between the veins, costae proximally with reddish brown flat lanceolate scales to 3.5 × 2.0 mm with finely cristate margins, midveins/costulae with similar scales smaller (to 2.0 × 1.2 mm) and weakly to strongly bullate.

Etymology.—The specific epithet refers to the mountain habitat (Greek *ὄρος* = *oros*, mountain, *ὄρειος* = *oreios* of the mountains) and the remote similarity to *Oreopteris* Holub, a genus of Thelypteridaceae.

Distribution and habitat.—*Cyathea oreopteroides* is known only from one locality in the Andes, occurring on exposed sandstone ridges under perhumid conditions, at 2000–2100 m.

Cyathea oreopteroides is another species in a long list of dwarf tree ferns from the sandstones in the Amotape-Huancabamba zone. *Cyathea bipinnatifida* (Baker) Domin, the most widespread of the pinnate-pinnatifid tree fern species in South America and a typical dweller of the understory, grows close to *C. oreopteroides* in comparable open vegetation. Some tree ferns respond to suboptimal growing conditions quite drastically in their morphology, e.g. to too much sun exposure with reduced size, denser hairy indument, either by increase of number of hairs or simply by contraction of laminar tissue, and fading of scale color. Finding both species in the same habitat and morphologically separable shows that *C. oreopteroides* is not a mere ecotype of *C. bipinnatifida* (trunks absent or very short and branched, usually less than 20 pinna pairs, many flat brown scales on lamina, and paraphyses short in *Cyathea oreopteroides* vs. presence of a discernible trunk, commonly more than 20 pinna pairs per frond, few small white bullate scales abaxially on the lamina, paraphyses weakly longer than sporangia in *C. bipinnatifida*).

Cyathea concordia B. León & R. C. Moran, from the Cordillera del Condor at 1800–2050 m, resembles *C. oreopteroides* by habit and the coloration and the firm texture of the dried lamina. Both species differ by absence or presence of the indusia (absent in *Cyathea oreopteroides* vs. present and sphaeropteroid in

C. concordia) and the hair distribution (hairs only on veins, and more abundant abaxially vs. hairs also between veins and equally abundant on both sides). *Cyathea palaciosii* R.C.Moran is also exindusiate and of the same size as *C. oreopteroides* but has softer laminar texture and almost no scales on the lamina. From all the afore mentioned species, *C. thelypteroides* (Cerro Tayu, Prov. Amazonas, Peru; Fig. 2E) differs in having at least weakly shiny laminae and petioles, the latter may be almost black in mature fronds and contrasting with the shiny brown petiole scales. It also lacks hairs on the laminae except for the leaf axes and midveins adaxially.

Not far to the east from *Cyathea oreopteroides* occurs *C. phegopteroides* at ca. 1000–1200 m in the area of Tarapoto, Peru. *Cyathea phegopteroides* has a similar firm laminar texture and scaly frond axes but it has longer, spreading hairs on the rhachis, and at least some of the petiole scales with a blackish center. Furthermore, it typically has basally decurrent laminae and short petioles, but some Ecuadorean plants lack this character. Given the small documented ranges of these dwarf species, the Ecuadorean material may turn out to be another distinct species after closer inspection.

Discussion

The Amotape-Huancabamba zone (Weigend, 2002, 2004; in the following as "AHZ"), also known as the Western Andean Portal (Antonelli *et al.,* 2009), marks the geological and biogeographical transition between the Northern and the Central Andes in southern Ecuador and northern Peru (Fig. 5). It is a low lying stretch of the cordillera with a varied topography on a mosaic of bedrocks and for thousands of kilometer north and south, is the only passage for humid air masses from the Amazon basin (Beck *et al.*, 2008). This creates many differently sized islands of cloud forests on mountain crests and windward slopes separated by dry valleys on the leeward sides. This habitat structure is believed to have fostered the evolution of the plethora of endemic plant and animal species known from this zone (Weigend, 2004; Barthlott *et al.*, 2005; Antonelli *et al.*, 2009). However, true Alpine zones, as the páramos of the Northern Andes and the púnas of the Central and Southern Andes, are completely absent (Luteyn and Churchill, 1999). While the northern and southern boundary of the zone is quite loosely defined, the dry river valley of the Río Huancabamba, extending into the lower Río Marañon valley, is a sharp boundary for many species coming from the north and south, widespread species and AHZ-endemics alike (Weigend, 2004); this line is sometimes referred to the Huancabamba depression in the strict sense (Weigend, 2004). To the west, the AHZ borders to dry forest of the Tumbesian biogeographic region (Linares-Palomino *et al.,* 2003) whereas to the east, it grades rather gently into Amazonian lowland forest. The simultaneous biogeographic separation and overlap in the AHZ results in high species richness at the local and regional scale (Barthlott *et al.,* 2005)

The location at the Abra Patricia (Prov. San Martín, Peru; Fig. 5), where the two new Peruvian species described herein were discovered, is exceptionally

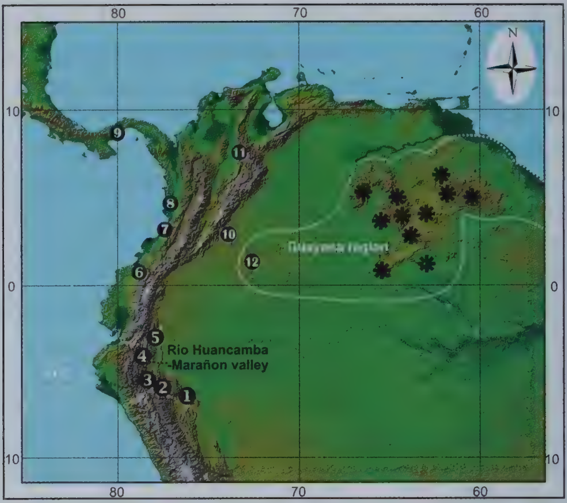

FIG. 5. Occurrence of *Cyathea* species of the *Hymenophyllopsis* clade (asterisks) on the tepuís of the Guayana region (*sensu* Berry and Riina, 2005); approximate locations with *Cyathea aterrima* (CATE), *Cyathea falcata* (CFAL), and/or *Pterozonium* sp. (PTRZ) as numbered dots: 1. Mt. Campana & Mt. Guayrapurima, near Tarapoto, Perú (CATE); 2. Abra Patricia & Morro de Moyobamba, Perú (CATE); 3. Perú, Cerro Tayu (CATE, PTRZ); 4. Río Nangaritza valley, Ecuador (PTRZ); 5. Cordillera del Cóndor & Cordillera de Cutucú, border Ecuador/Perú (PTRZ); 6. Prov. Esmeraldas, Ecuador (CATE); 7. Dagua valley Colombia (CATE, CFAL); 8. Cerro Mecana, Serranía del Baudó, Colombia (CATE); 9. Prov. Coclé, Panama (CFAL); 10. Serranía de Macarena, Colombia (CATE); 11. Mesa de los Santos, Colombia (CATE); 12. Hoya Soplador, Colombia (CATE, PTRZ). AHZ = Amotape-Huancabamba zone (*sensu* Weigend, 2004), area between dashed lines west of the Andes often treated as part of the Tumbesian region (Linares-Palomino *et al.*, 2003). Basemap © wikicommons, modified by M. Lehnert.

rich in tree fern species. At least 20 species of Cyatheaceae (plus a putative hybrid) were recorded on soils over sandstone bedrock along a trail spanning less than 2 km distance and 100 m difference in elevation. On the opposite side of the valley, which has a similarly dense cover of vegetation but on limestone, only one member of the family, *Cyathea crenata* (Sodiro) C.Chr., was seen in small numbers. This emphasizes the importance of sandstones for the diversity and endemism of scaly tree ferns in the Neotropics. In the Paleotropics, however, this substrate preference is not as strong. Sandstones do not stand out among other substrates that usually harbor many narrow endemics, such as ophiolithic (serpentine) and ultramafic substrates. Limestone areas in the Philippines, on Seram (Indonesia), and on New Guinea seem to have a considerable number of tree ferns (mainly *Alsophila*), often with localized endemics (e.g., Kato, 1990; Takeuchi, 2007).

The sandstones of the northern Andes, including the AHZ, are derived from Cretaceous deposits that have been gradually exposed, eroded and often covered by new sediments in the wake of the Andean uplift (McCourt *et al.*, 1984). This type of sandstone can be found on both sides of the northern Andes and often forms smaller promontory mountain ranges separated from the main cordillera (e.g., the Colombian Serranía de Macarena and the Ecuadorian Cordillera de Cutucú; Fig. 5). These Andean sandstones are of a different age and origin than the sandstones of the Guayana shield, which are Proterozoic deposits more than 542 My old (Berry and Riina, 2005), yet both are often shaped today into similar table mountains (Venezuela: "tepuís") and show comparable vegetation structure, with stunted forests and scrub vegetation on the upper plateaus and lush montane forests on the slopes (Barthlott *et al.*, 2005). Starting from the foothills, which share most species with the adjacent lowland vegetation, there is an increasing degree of endemism towards the upper plateaus of the table mountains (Barthlott *et al.*, 2005). Due to larger size, wider area of occupancy, and older age, the tepuís of the Guayana shield are more distinct in their vegetation structure and more easily characterized than the Andean counterparts and boast a unique composition of genera that have radiated into many endemic species, often with few or no species shared between the distinct tepuís (Berry and Riina, 2005). Yet some of the typical tepuí elements found their way to the Andean sandstone areas, bridging a distance between 750 and 1500 km by air. These include Angiosperms like *Purdiaea nutans* Planch. (Cyrillaceae; Anderberg and Zhang, 2002) and cryptogamic plants like liverworts (Pleuroziaceae: *Pleurozia paradoxa* (J.B.Jack) Schiffn., Mandl *et al.*, 2008) and ferns (Pteridaceae: *Pterozonium brevifrons* (A.C.Sm.) Lellinger, *P. reniforme* (Mart.) Fée; Smith in Steyermark *et al.*, 1995). Among the scaly tree ferns, however, most connections at the species level seem to be vice versa, with relatively widespread species of Andean radiations also occurring on the slopes of some tepuis (Smith in Steyermark *et al.*, 1995), e.g., *Cyathea bipinnatifida* (see discussion under *C. oreopteroides*). *Cyathea* that appear to be ancestrally from the Guayana shield have not been recorded in the Andes, and their similarity to some Andean and Mesoamerican species may be due to parallel evolution. The true relationships have yet to be tested, e,g., between the simply pinnate species *C. williamsii* (Maxon) Domin (Panama) and *C. maguirei* (R.M.Tryon) Lehnert (Venezuela), and the bipinnate species *C. bipinnata* R.M.Tryon (Colombia, Ecuador), and *C. intramarginalis* (P.G.Windisch) Lellinger (Venezuela).

Most noteworthy are the tepuí endemics formerly treated as a separate monotypic family Hymenophyllopsidaceae (Smith in Steyermark *et al.*, 1995): the eight currently recognized *Cyathea* species of the *Hymenophyllopsis* clade (subgenus *Hymenophyllopis* sensu Christenhusz, 2009) are apparently adapted to different substrate types (i.e., lithophytic, epiphytic, terrestrial), water regimes (i.e., dry ledges to seepage areas), and levels of light exposure, so that two or more species can be found on one tepuí, and most species occur on several tepuís, sometimes with populations separated by up to 300 km distance by air. Besides their thin fronds that look like those of filmy ferns (Hymenophyllaceae), species of the *Hymenophyllopsis* clade are further characterized by reduced size (fertile plants including fronds and rhizome just 10–30 cm long) and small, narrow, thin scales that may grade into hairs. In the Andean sandstone areas under conditions where species of the *Hymenophyllopsis* clade may be expected, *Cyathea aterrima* (Hook.) Domin is usually found. This species is also characterized by narrow, thin scales that grade into hairs, and by a pronounced pedomorphism: fertile plants may have bipinnate-pinnatifid fronds over 2 m long and thick trunks exceeding 2 m in height, but most are trunkless with much smaller, less divided fronds (Lehnert, 2016). One may see in this example how the nutrient deficient sandstone substrate has formed the strongly aberrant physiognomy of the *Hymenophyllopsis* clade.

In the sandstone areas on both sides of the northern Andes, *Cyathea aterrima* and *Pterozonium brevifrons* are relatively widespread (Fig. 5) and often found together on vertical cliffs. Other *Cyathea* species adapted to this habitat have more restricted distributions and are often known only from a single locality. For example, *C. falcata* (Mett. ex Kuhn) Domin is relatively widespread in the Chocó of eastern Panamá and western Colombia on wet cliff faces, but the putative next relatives have smaller ranges: *Cyathea brucei* Lehnert is found only along the Río Nangaritza in southern Ecuador, and *C. haughtii* (Maxon) R.M.Tryon is known only from one locality from northern Colombia.

The species group around *Cyathea bipinnatifida*, characterized by pinnate-pinnatifid fronds and thin, often decumbent trunks, shows a pattern of decreased size and increased geographic restriction from relatively tall understory plants of talus forest (*C. bipinnatifida*: Andes of Colombia to Bolivia; *C. decorata* (Maxon) R.M.Tryon: Chocó, western Colombia), over smaller plants of open ridge-top forest (*C. alstonii* R.M.Tryon: Serranía de Macarena, Colombia; *C. peladensis*: eastern Andes of southern Colombia to northern Peru) to ± trunkless plants of shrubby crest vegetation (*C. oreopteroides,* Abra Patricia, Peru; *C. phegopteroides,* Mt. Guayrapurima, Peru) and rock faces (*C. concordia*, Cordillera del Cóndor, Peruvian-Ecuadorian border; *C. palaciosii*, Río Nangaritza. southern Ecuador; *C. thelypteroides*, Cerro Tayu, northern Peru). From this list, the last five species are endemic to the AHZ zone, and *C. peladensis* is defined in its distribution to the south by the Huancabamba depression (Weigend, 2004).

The rugged topography of the Amotape-Huancabamba zone harbors many still unexplored sandstone peaks, where further narrowly endemic tree fern species may await discovery.

ACKNOWLEDGMENTS

We thank our colleagues Gloria Calatayud, Marylaure de la Harpe, Adriana Hernandez, Jürgen Homeier, Michael Kessler, Christian Lexer, Oriane Loiseau, Julian Mostacero, Ingrid Olivares, Margot Paris, Nicolas Salamin, Leonardo Versieux and Anna Weigand for their company and help in the field. Asunción Cano, Ellúz Huaman and Blanca León kindly organized the collection permit for Peru. Collection permits were kindly issued by the Ministerio del Ambiente Ecuador (N° 036-2014-FLO-DP/MAE) and INRENA Peru (Resolución Directoral N° 0449-2010-DGFFS-DGEFFS).

Field trips that contributed to our findings were financed through the German Science Foundation (DFG; grant LE 1826/4) and the research project POPCORN (Using Population genomics, Phylogenetics, and Community Ecology for understanding Radiations in Neotropical mountains) funded by the Swiss National Fund (grant SNF-310030L_146906).

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American Fern Journal 106(3):191–205 (2016)
Published on 19 October 2016

Oleandra (Oleandraceae) in the Brazilian Atlantic Forest

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Abstract.—We present a taxonomic treatment of the genus *Oleandra* in the Brazilian Atlantic Forest. Five endemic species and one putative hybrid are recognized: *O. australis, sp. nov.* (endemic to the state of Paraná), *O. baetae* (endemic to state of Minas Gerais), *O. brasiliana, sp. nov.* (widespread along the Brazilian Atlantic Forest), *O. hirta* (endemic to the state of Rio de Janeiro), *O. quartziticola, sp. nov.* (endemic to the state of Espírito Santo), and *O. baetae* × *O. brasiliana* (from places of sympatry). The widely used name "*Oleandra articulata*" does not apply to the Brazilian Atlantic Forest species. All species are described, illustrated, and mapped.

Key Words.—endemic ferns, hybrids, south and southeastern Brazil, taxonomy

Resumo (Portuguese).—Apresenta-se o tratamento taxonômica do gênero *Oleandra* para a Floresta Atlântica brasileira. Ali, cinco espécies endêmicas e um suposto híbrido são reconhecidos: *O. australis, sp. nov.* (endêmica ao estado do Paraná), *O. baetae* (endêmica ao estado de Minas Gerais), *O. brasiliana, sp. nov.* (amplamente distribuída na Floresta Atlântica), *O. hirta* (endêmica ao estado do Rio de Janeiro), *O. quartziticola, sp. nov.* (endêmica ao Estado do Espírito Santo) e *O. baetae* × *O. brasiliana* (em locais de simpatria). O nome "*Oleandra articulata*", amplamente utilizado, não se aplica às espécies da Floresta Atlântica brasileira. Todas as espécies são descritas, ilustradas e mapeadas.

Oleandra Cav. belongs to the pantropical, monogeneric family Oleandraceae (*sensu* Smith *et al.,* 2008), which is the sister group to the clade comprising Davalliaceae and Polypodiaceae (Schneider *et al.,* 2004; Schuettpelz and Pryer, 2008; Tsutsumi and Kato, 2006). The main synapomorphy of these three families is the articulation of the leaves on phyllopodia (mostly lost in the grammitid lineage), which functions as an abscission zone (*e.g.,* Addicott, 1982). However, the phyllopodia in Oleandraceae are "stipe-like", while in the other two families they are "rhizomatous" (Hovenkamp and Ho, 2012).

Oleandra is mainly characterized by creeping rhizomes clothed with peltate scales, leaves articulated to pronounced stipe-like phyllopodia, simple laminae, parallel veins, and sori protected by reniform indusia (Hovenkamp and Ho, 2012; Smith *et al.,* 2008). Although the generic circumscription has been stable (*e.g.,* Copeland, 1947; Kramer, 1990; Smith *et al.* 2008; Tryon and Tryon, 1982), species delimitation has been problematic, both in the New and Old Worlds. Species number estimates have ranged from ca. 15–20 (*e.g.,* Tryon, 1997, 2000; Hovenkamp and Ho, 2012; Xianchum and Hovenkamp, 2013) to ca. 40–50 (Copeland, 1947; Mickel and Smith, 2004; Proctor, 1989; Smith *et al.,* 2008). For the New World, the estimates have ranged from three species (Copeland, 1947), to four (Tryon, 1997), to six (Tryon and Tryon, 1982), to eight (Mickel and Smith, 2004), up to 11 (Maxon, 1914).

The two taxonomic revisionary works on *Oleandra* from the Americas are those by Maxon (1914) and Tryon (1997), which conflict with each other. Maxon (1914) presented a narrow species concept, recognizing 11 species for the Americas. On the other hand, Tryon (1997) used a broader circumscription for widespread species, and he reduced the New Word species to four. For the Brazilian Atlantic Forest, Maxon (1914) recognized *O. articulata* (Sw.) C.Presl and *O. hirta* Brack., while Tryon (1997) accepted only *O. articulata* there (placing *O. hirta* in synonymy). Recently, Salino and Almeida (2009) revalidated *O. articulata* and *O. hirta*, but Sylvestre (2010) and Prado *et al.* (2015) recognized *O. articulata* (with *O. hirta* in synonymy) and *O. baetae* Damazio.

The goal of the present paper is to re-evaluate the taxonomy of *Oleandra* occurring in the Brazilian Atlantic Forest, based on morphological study of specimens deposited in Brazilian herbaria, type specimens, geographic distributions, and *in situ* observations.

Material and Methods

We analyzed ca. 130 specimens of *Oleandra* (including types) from the following herbaria: CESJ, MBM, OUPR, RB, SP, SPF, UPCB, and VIC. We also observed images of types from K, P, and US herbaria, which are available online at REFLORA (http://reflora.jbrj.gov.br/jabot/herbarioVirtual/ConsultaPublicoHVUC/ConsultaPublicoHVUC.do), JStor Global Plants (https://plants.jstor.org/), and Smithsonian National Museum of Natural History (http://collections.nmnh.si.edu/search/botany/) websites.

We studied living populations *in situ,* in order to verify local morphological variation, and to collect representative specimens. We found *Oleandra brasiliana* Schwartsb. & J.Prado in two localities (Santo Antônio do Itambé, MG, and Maranguape, CE)*, O. baetae* in two localities (Lima Duarte and São João del Rei, MG), and *O. quartziticola* Schwartsb. & J.Prado in one locality (Vargem Alta, ES).

The distribution maps were drawn using the software Diva-Gis (Hijmans 2015). For the materials without geographical coordinates, we estimated them using Google Earth (available from https://www.google.com/earth/). For background, we used the Brazilian biomes layer, available at IBGE website (www.ibge.gov.br), and the Brazilian administrative areas layer, available at Diva-Gis website (www.diva-gis.org). We manually changed the Brazilian Atlantic Forest to gray, leaving all the other biomes in white.

Morphological terms (including organ names and lamina shapes) follow Lellinger (2002) and Hovenkamp and Ho (2012). Our generic description was especially based on the Brazilian Atlantic Forest taxa, in order to eliminate repetition of common features among species. Species descriptions present the morphological characters with taxonomic value. We did not personally analyze any specimens of *Oleandra hirta,* which is known only from the type collection. Brackenridge (1854) provided a detailed description and illustration of the species, and the images online are high resolution showing excellent detail. Thus, we could include it in our treatment. Unfortunately, we could not describe the rhizome scales nor the type of laminar hairs (*i.e.,* either catenate and/or glandular).

We here propose a new and more restricted circumscription of *Oleandra articulata.* To reach this circumscription, we started with the descriptions provided by Proctor (1977 [as "*O. nodosa* (Willd.) C.Presl"], 1985, 1989), Palacios-Ríos (1995), and Mickel and Smith (2004), and followed with study of specimens from around the type locality (especially from Martinique, Jamaica, La Guadaloupe, and Cuba). We also analyzed Amazonian plants previously ascribed to "*O. articulata*". The specimens examined are housed in MBM, RB, and SP herbaria.

RESULTS AND DISCUSSION

Based on morphological comparisons and geographical distributions, we propose a new species concept for the Brazilian Atlantic Forest species of *Oleandra*, which is narrower than that of Maxon (1914). There, five endemic species and one putative hybrid occur: *Oleandra australis* Schwartsb. & J.Prado (endemic to the state of Paraná), *O. baetae* (endemic to state of Minas Gerais), *O. brasiliana* (widespread along the Brazilian Atlantic Forest), *O. hirta* (endemic to the state of Rio de Janeiro), *O. quartziticola* (endemic to the state of Espírito Santo), and a putative hybrid involving *O. baetae* and *O. brasiliana* (from areas of sympatry between these two species).

The widely used name "*Oleandra articulata*" does not apply to the Brazilian Atlantic Forest species, in its strict sense (see comments of *O. brasiliana* below). Based on our analyses, *O. articulata* is probably restricted to the Antilles, Mesoamerica, and Mexico, and does not occur in South America (plants from the Amazon previously identified as "*O. articulata*" are also different).

Except for *Oleandra brasiliana,* which is glabrescent and sometimes seems fully glabrous, the other species from the Brazilian Atlantic Forest all have pubescent laminae. It is important to note that they all bear catenate and multicellular glandular hairs. The glandular hairs are easily detected in living specimens (*i.e.,* the laminae are sticky), but they are not easily observed in dried specimens. The capitate head (the gland) is very small, and is best visualized under a minimum magnification of 100×. The same magnification is required to reliably observe the glandular cilia of the rhizome scales.

Taxonomic Treatment

Oleandra Cav., Anales Hist. Nat. 2:115. 1799. *Aspidium* subg. *Oleandra* (Cav.) Splitg., Tijdschr. Natuurl. Gesch. Physiol. 7:411. 1840. Type: *Oleandra neriiformis* Cav. (as "*neriformis*"). Figs. 1, 2.

For additional synonymy see Hovenkamp and Ho (2012).

Plants epiphytic or rupestrial. *Rhizomes* long-creeping, dorsi-ventral, light brown to blackish (commonly greenish *in vivo*), pruinose or not, 1–4 mm diam., with rhizophore-like roots; *scales* spreading, bicolorous at base (darker only at the attachment point, orange in the remaining parts) or entirely bicolorous (centrally blackish, orange at the margins), sparsely to densely glandular-ciliate. *Phyllopodia* 5–45 mm long, stipe-like. *Fronds* sparse, monomorphic, articulated to phyllopodia, 9–55 cm long; *petioles* 0.3–7(–10) cm long, stramineous to dark brown, or greenish; *laminae* simple, linear, elliptical or obovate; *midribs* with catenate and/or glandular hairs, or glabrescent (sometimes appearing glabrous); *veins* free or rarely partly anastomosing, with or without catenate and/or glandular hairs; *laminar tissue between the veins* with or without catenate and/or glandular hairs; *laminar margins* cartilagineous, with or without catenate and/or glandular hairs; *sori* infra-medial to medial, in one irregular row between midribs and laminar margins; *indusia* reniform or orbicular-reniform, glabrous or with hairs; *spores* monolete, light to dark brown.

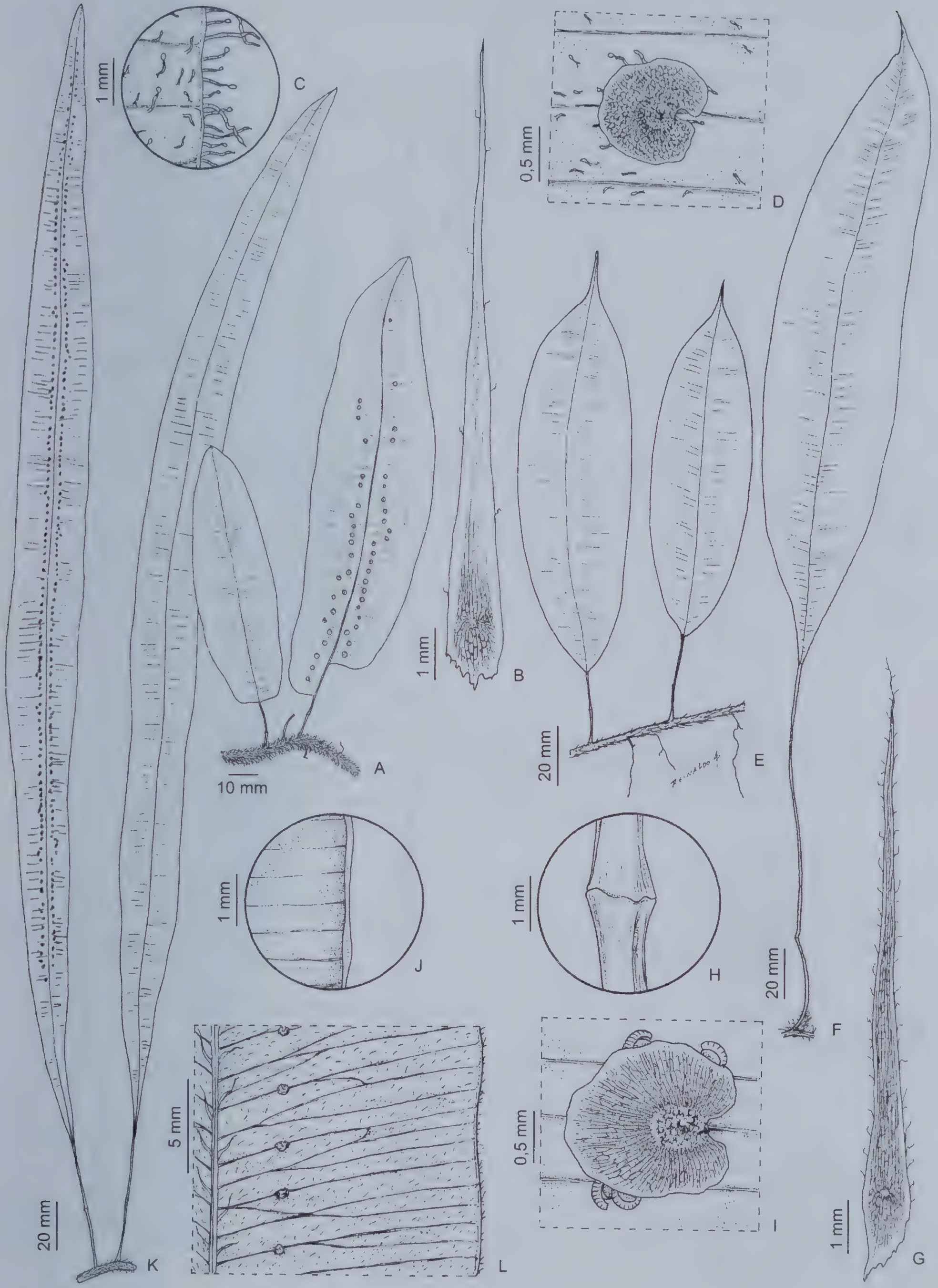
Key to Species of *Oleandra* from the Brazilian Atlantic Forest

1. Laminae linear; veins partly anastomosing . . . . *O. hirta*
1. Laminae elliptical or obovate; veins free
    2. Both sides of midribs and laminar margins glabrescent, with scattered hairs (especially in the laminar apices), or appearing glabrous . . . . *O. brasiliana*
    2. Both sides of midribs and laminar margins pubescent throughout
        3. Rhizome scales bicolorous at base (darker only at the attachment point, orange in the remaining parts); laminar tissue between the veins abaxially pubescent *O. baetae*
        3. Rhizome scales entirely bicolorous (centrally blackish, orange at the margins); laminar tissue between the veins abaxially glabrous
            4. Laminar tissue between the veins adaxially glabrous . . . . *O. australis*
            4. Laminar tissue between the veins adaxially pubescent . . . . *O. quartziticola*

Oleandra australis Schwartsb. & J.Prado, ***sp. nov.*** Type.—BRAZIL. **Paraná:** Antonina, Reserva Natural do Cachoeira, SPVS, 25°14′28"S, 48°40′23"W, 350 m, 25 Jun 2007, *P.H. Labiak & F.B. Matos 3941* (holotype: UPCB!; isotypes: MBM!, NY, image seen, SP!, VIC!). Figs. 2C–F, 3.

→

Fig. 1. A–D. *Oleandra baetae*. A. Habit. B. Rhizome scale. C. Detail of lamina abaxially and laminar margin. D. Indusium. E–J. *O. brasiliana*. E. Habit. F. Habit. G. Rhizome scale. H. Articulation between phyllopodium and petiole. I. Indusium. J. Detail of lamina abaxially and laminar margin (from a mature glabrescent specimen). K, L. *O. hirta*. K. Habit. L. Detail of partly



anastomosing veins, abaxially. (A–C from *Schwartsburd 3515*; D from *Baeta 258*; E, H–J from *Schwartsburd 2516*; F from *Duarte 9767*; G from *Matos 377*; K, L from *Wilkes s.n.*).

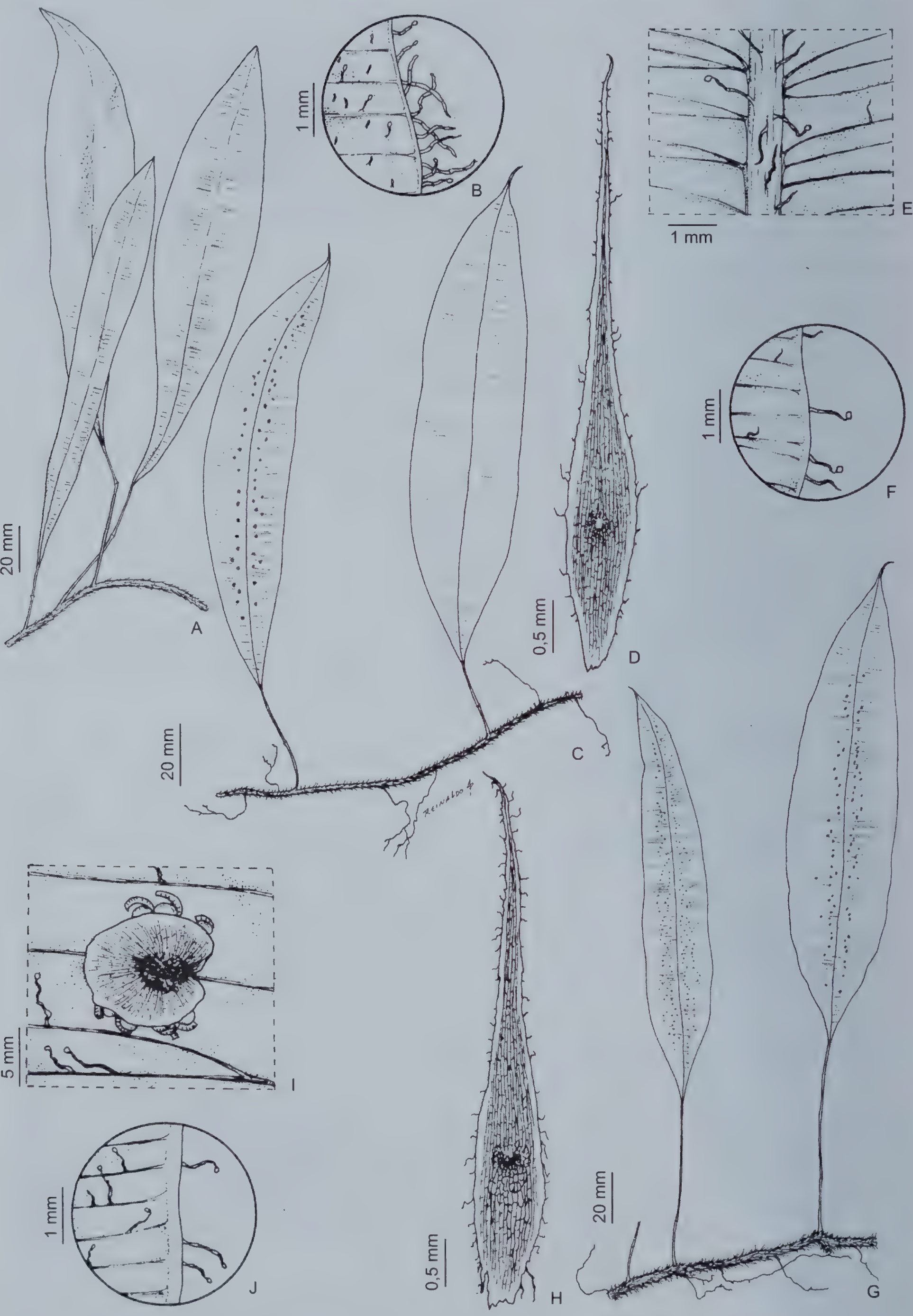


FIG. 2. A–B. *Oleandra baetae* × *O. brasiliana* A. Habit. B. Detail of lamina abaxially and laminar margin. C–F. *O. australis*. C. Habit. D. Rhizome scale. E. Detail of midribs, abaxially. F. Detail of lamina abaxially and laminar margin. G–J. *O. quartziticola*. G. Habit. H. Rhizome scale. I.

Rhizome scales bicolorous; laminae elliptical; midribs, veins, and laminar margins with catenate and glandular hairs; laminar tissue between the veins glabrous on both surfaces.

Plants epiphytic. *Rhizomes* light brown, not pruinose, 1–2 mm diam.; *scales* bicolorous, centrally dark brown, orange at the margins, densely glandular-ciliate. *Phyllopodia* (8–)10–35 mm long. *Fronds* 12–20 cm long; *petioles* 1–4 cm long, greenish; *laminae* elliptical, cuneate at base, aristate at apex, 11–18 × 2.5–4.5 cm; *midribs* with 3–7(–10)-celled catenate hairs, and 3–7(–10)-celled glandular hairs on both sides; *veins* free, glabrescent on both sides, with sparse catenate and glandular hairs; *laminar tissue between the veins* glabrous on both sides; *laminar margins* with catenate and glandular hairs; *sori* infra-medial to medial; *indusia* orbicular-reniform, bicolorous, dark brown in the middle, light brown in the margins, glabrous; *spores* light brown.

Distribution and ecology.—Endemic to the state of Paraná, southern Brazil; ca. 350 m elev. (Fig. 3).

Etymology.—The specific epithet refers to the austral distribution of the species.

Oleandra australis is the southernmost species of *Oleandra* in the Americas, and it is known only from the type collection. *Oleandra australis* is characterized by entirely bicolorous rhizome scales, elliptical laminae, catenate and glandular hairs present on both sides of midribs and laminar margins, glabrous laminar tissue between the veins, and bicolorous indusia (Figs. 2C–F).

Oleandra baetae Damazio, Bull. Herb. Boissier, ser. 2, 6:892. 1906. Type.—BRAZIL. **Minas Gerais:** Serra do Frasão, 1906, *A. Baeta 258* (holotype: OUPR!-10824 [ex Herb. Damazio 460]; isotypes: P-00630964, image seen, P-00630965, image seen). Figs. 1A–D, 3.

Oleandra nodosa (Willd.) C.Presl var. *magalhaesii* Christ *in* Schwacke, Pl. Nov. Mineir. 2:29. 1900, as "*Magalhaesi*". Type.—BRAZIL. Minas Gerais: Serra do Ibitipoca, Sep 1897, *H. Magalhães 2259* (holotype: P-00630963, image seen).

Plants rupestrial. *Rhizomes* dark brown, pruinose, 2–4 mm diam.; *scales* bicolorous at base (darker only at the attachment point, orange in the remaining parts), sparsely glandular-ciliate. *Phyllopodia* 5–15 mm long. *Fronds* 9–15(–22) cm long; *petioles* 3–5 mm long, stramineous; *laminae* elliptical, truncate or inequilateral at base, acute or obtuse at apex, 8–14(–21) × 3–4.5 cm; *midribs* with 6–10-celled catenate hairs and 3–5-celled glandular hairs on both sides; *veins* free, with catenate and glandular hairs on both sides; *laminar tissue between the veins* with catenate and glandular hairs on both sides; *laminar margins* with catenate and glandular hairs; *sori* infra-medial;

←
Indusium. J. Detail of lamina abaxially and laminar margin. (A, B from *Badini s.n.*; C–F from *Labiak 3941*; G–J from *Schwartsburd 3522*).

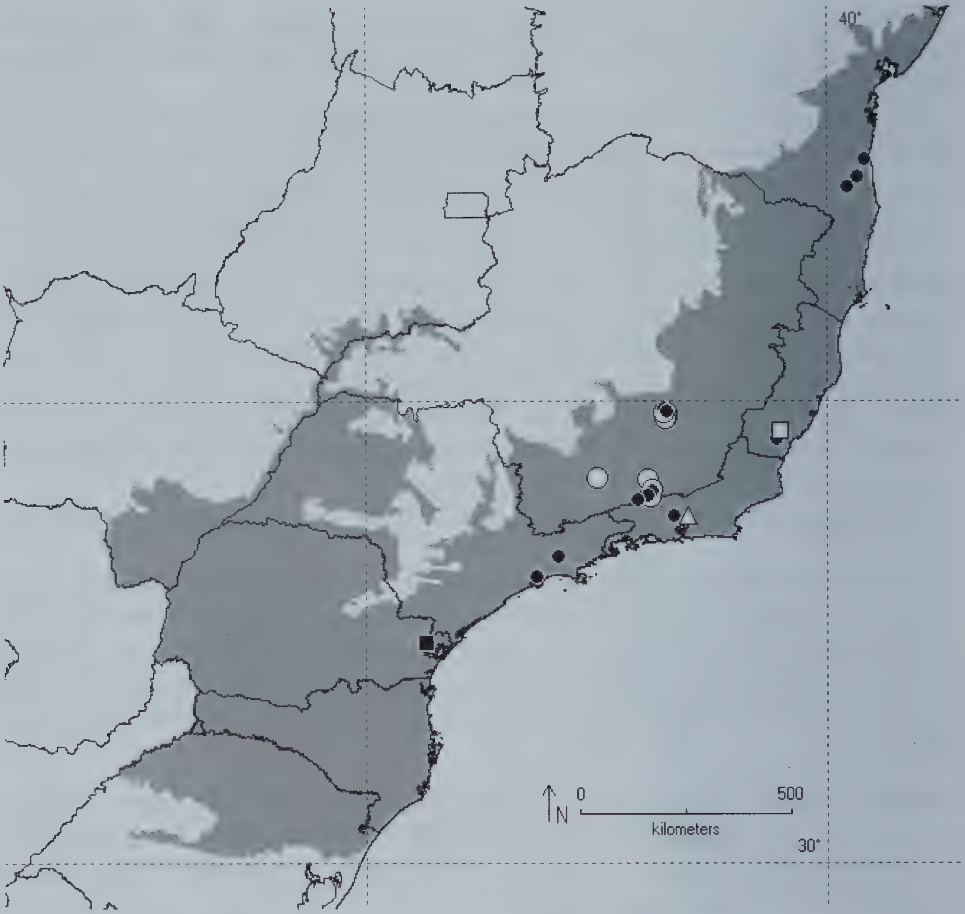


FIG. 3. Distribution of *Oleandra* species in the Brazilian Atlantic Forest (in gray): *O. australis* (black square), *O. baetae* (white circles), *O. brasiliana* (black circles), *O. hirta* (white triangle), and *O. quartziticola* (white square).

indusia orbicular-reniform, light brown, with sparse glandular hairs or glabrescent; *spores* reddish-brown to dark brown.

Distribution and ecology.—Endemic to the state of Minas Gerais; ca. 1,000–1,500 m elev. (Fig. 3).

SPECIMENS EXAMINED.—BRAZIL. **Minas Gerais:** Campo Grande, 1942, *J. Badini 4106* (OUPR); Campo Grande, 19 May 1975, *J. Badini 22321* (OUPR); Lima Duarte, Conceição do Ibitipoca, Parque Estadual Florestal do Ibitipoca, 5 Dec 1992, *J.E.Z. Oliveira 8* (CESJ); Lima Duarte, Conceição do Ibitipoca, Parque Estadual Florestal do Ibitipoca, 1 May 1993, *R.F. Novelino et al. 1020* (CESJ); Lima Duarte, Conceição do Ibitipoca, Parque Estadual Florestal do Ibitipoca, 15 May 1993, *J.E.Z. Oliveira et al. 267* (CESJ); Lima Duarte, Conceição do Ibitipoca, Parque Estadual Florestal do Ibitipoca, 1,360 m, 29 May 1993, *J.E.Z.*

Oliveira et al. 296 (CESJ); Lima Duarte, Conceição do Ibitipoca, 21°42′S, 43°53′W, 1,300 m, 9 Aug 2015, *P.B. Schwartsburd & R. Santana Milagres 3515* (SP, VIC); Lima Duarte, Serra Negra, 21°55′49.9"S, 43°46′43.1"W, 986 m, 15 Nov 2008, *F.R.G. Salimena & P.H. Nobre 2757* (CESJ); Ouro Preto, Andorinhas, 21 Jun 1974, *J. Badini 21702* (OUPR); Ouro Preto, Cachoeira Andorinhas, 1940, *E.V. Miranda 63* (CESJ, SPF); Ouro Preto, Camarinhas, 9 May 1974, *J. Badini 22058* (OUPR); Ouro Preto, 19 Sep 1989, *L. Krieger 24551* (CESJ); Rio Preto, Ribeirão Santana, 22 Mar 1997, *R.B. Tostes* (CESJ-29779, VIC-47056); São João del Rei, Serra do Lenheiro, 11 Jan 1999, *J. Prado et al. 996* (NY, SP); São Thomé das Letras, 13 Jul 1950, *A.C. Brade & A.P. Duarte 20465* (RB); São Thomé das Letras, 1,250 m, 13 Jul 1950, *A.C. Brade 3451* (CESJ); São Thomé das Letras, 14 Jul 1954, *A.P. Duarte 3834* (RB-on 2 sheets, VIC); São Thomé das Letras, 13 Oct 1984, *L. Krieger & A. Pavan 20641* (CESJ); São Thomé das Letras, 1300 m, 1 Jul 1987, *L.S. Kinoshita-Gouvêa et al. 19126* (SP, UEC-*n.v.*, VIC); São Thomé das Letras, Gruta São Tomé, 21°43′23"S, 44°59′09"W, 1278 m, 11 Jan 2016, *P.S.P. Sampaio & A.F.P. Sampaio 1355* (HUSC, VIC). Serra do Frasão, 1942, *J. Badini 4104* (OUPR); Serra de Ouro Preto, Mar 1907, *L. Damazio 380* (OUPR [on 2 sheets]); Serra de Ouro Preto, 9 May 1917 [or 1907?], *L. Damazio s.n.* (RB-36235 [on 2 sheets]); Serra de Ouro Preto, s.d., *J. Badini 2610* (CESJ); Serra do Frasão, 12 Mar 1907, *L. Damazio s.n.* (RB-171642); Tiradentes, Serra de São José, 21°5′S, 44°10′W, 1,250 m, 24 Sep 1989, *R.J. Válka Alves & J. Holbek 700* (SP, SPF); Locality unknown, s.d., *L. Krieger 20931* (CESJ).

In the protologue of *Oleandra nodosa* var. *magalhaesii*, Christ (1900) cited the type was collected in "Serra do Itatiaia." A detailed examination of the label of the type (*Magalhães 2259* [P-00630963]) lead us to conclude he misread the locality. The correct type locality is Serra do Ibitipoca.

Oleandra baetae is endemic to the state of Minas Gerais, especially in the mountain regions of the south and southeast. It is characterized by rhizome scales bicolorous at base (darker only at the attachment point, orange in the remaining parts), short petioles (3–5 mm long), elliptical laminae, which are truncate or inequilateral at the base, catenate and glandular hairs conspicuously present on all laminar regions, and light brown indusia with glandular hairs (Figs. 1A–D).

Besides not overlapping in geographic range, *Oleandra baetae* differs from *O. hirta* by having fronds 9–15(–22) cm long (*vs.* 50–55 cm long), phyllopodia 5–15 mm long (*vs.* 25–42 mm), elliptical laminae (*vs.* linear), with truncate or inequilateral base (*vs.* long-attenuate base), and free veins (*vs.* partly anastomosing) (Figs. 1A–D *vs.* 1K, L; see also Brackenridge, 1854:t. 29).

Oleandra brasiliana Schwartsb. & J.Prado, ***sp. nov.*** TYPE.—BRAZIL. **Bahia:** Arataca, Serra do Peito de Moça, RPPN Caminho das Pedras, 15°10′25"S, 39°20′30"W, 950 m, 6 Aug 2006, *P.H. Labiak et al. 3652* (holotype: SP!; isotypes: NY, image seen, UPCB!). Figs. 1E–J, 3.

Rhizome scales bicolorous; laminae elliptical to obovate; midribs and laminar margins glabrescent, with scattered catenate and glandular hairs;

veins glabrous; laminar tissue between the veins abaxially glabrous, adaxially glabrescent.

Plants rupestrial or epiphytic. *Rhizomes* light brown to blackish, not pruinose, 1.5–2.5 mm diam.; *scales* bicolorous, centrally black, orange at the margins, moderate to densely glandular-ciliate. *Phyllopodia* 10–40 mm long. *Fronds* 12–35 cm long; *petioles* 3–7(–10) cm long, shining dark brown or greenish-brown; *laminae* generally obovate, rarely elliptical, cuneate at base, aristate at apex, 10–30 × (2–)4.5–7 cm; *midribs* glabrescent on both sides, with scattered 2–5-celled catenate hairs and 2–5-celled glandular hairs, sometimes seeming glabrous; *veins* free, glabrous on both sides; *laminar tissue between the veins* abaxially glabrous, adaxially glabrescent, with scattered catenate and glandular hairs; *laminar margins* glabrescent, with scattered catenate and glandular hairs, especially common at the laminar apices; *sori* infra-medial to medial; *indusia* orbicular-reniform, dark brown, glabrous; *spores* light brown.

Distribution and ecology.—Widespread along the Brazilian Atlantic Forest, from the state of Bahia to São Paulo, with a disjunct population in Ceará; from 600 to 1,200 m elev. (Fig. 3).

Etymology.—The specific epithet refers to the distribution of the species; *Oleandra brasiliana* is the most widespread of the Brazilian Atlantic Forest species.

Specimens Examined.—BRAZIL. **Bahia**. Arataca, 15°10′25"S, 39°20′30"W, 1,000 m, 20 Jan 2007, *A.M. Amorim et al. 6701* (UPCB, RB); Arataca, 15°10′25"S, 39°20′30"W, 1,000 m, 13 Apr 2007, *F.B. Matos et al. 1355* (SP, UPCB); Arataca, 15°10′12"S, 39°20′17"W, 940 m, 13 Nov 2011, *L. Daneu et al. 581* (RB); Barro Preto, 14°46′13"S, 39°12′10"W, 600–900 m, 8 Feb 2005, *F.B. Matos et al. 377* (MBM, UPCB); Camacã, 15°23′30"S, 39°33′55"W, 835 m, 13 Feb 2005, *F.B. Matos et al. 440* (UPCB); Camacã, 15°23′30"S, 39°33′55"W, 835 m, 9 Jul 2005, *F.B. Matos et al. 628* (UPCB); Camacã, 15°23′35"S, 39°33′53"W, 850 m, 11 Aug 2006, *P.H. Labiak et al. 3710* (SP, UPCB). **Ceará**: Maranguape, 3°54′05"S, 38°43′12"W, 900 m, 10 Apr 2011, *P.B. Schwartsburd & J.A.P. Araújo 2516* (EAC, NY, SP, VIC). **Espírito Santo:** Cachoeiro do Itapemirim, Vargem Alta, Morro de Sal, 21 Aug 1948, *A.C. Brade 19324* (RB-on 2 sheets); Cachoeiro do Itapemirim, Vargem Alta, Morro de Sal, 21 Aug 1948, *A.C. Brade 3452* (CESJ); Cachoeiro do Itapemirim, Vargem Alta, 3 May 1966, *A.P. Duarte 9767* (MBM, RB-on 2 sheets, VIC). **Minas Gerais:** Mariana, Serra do Frasão, km 7, 19 Sep 1989, *R.F. Novelino et al. s.n.* (CESJ-24127, VIC-47057); Monte Verde de Cima, Serra Negra, 9 Mar 1991, *M. Brugger & H.G. Souza s.n.* (CESJ-24711 [on 2 sheets], VIC-47054); Santa Bárbara do Monte Verde, 21°57′55"S, 43°49′51"W, 1,200 m, 24 Apr 2004, *J.P.S. Condack 135* (RB, VIC); Rio Preto, Serra Negra, 17 Mar 2007, *N.L. Abreu et al. 151* (CESJ, MBM); Rio Preto, Serra Negra, Sep 2011, *R.J.V. Alves & N.L. Abreu 8751* (CESJ, VIC); Rio Preto, Vilarejo do Funil, 21 May 2004, *F.R.G. Salimena et al. 1286* (CESJ); Santa Rita do Jacutinga, 17 Apr 1992, *L. Krieger s.n.* (CESJ-29460, VIC-47055); Santo Antônio do Itambé, Serra do Espinhaço, 18.45475° S, 43.33445°W, 865 m, 14 Jan 2010, *E. Schuettpelz et al. 1415* (MO, SP); Serra do Capanema, 1943, *J. Badini 4239* (OUPR); Serra do

Frasão, 1906, *A. Baeta 259* (OUPR); Serra do Frasão, 26 Mar 1907, *L. Damazio et al. 381* (OUPR-on 2 sheets); Serra do Frasão, 1907, *L. Damazio s.n.* (RB-36236); Serra do Frasão, 1934, *J. Badini & P. Lisboa e Costa s.n.* (OUPR-10833 [on 2 sheets]); Serra do Frasão, 1934, *J. Badini 253* (RB); Serra do Frasão, 1943, *J. Badini 4238* (OUPR); Serra do Frasão, 19 Sep 1989, *R.F. Novelino et al. 706* (OUPR). **Rio de Janeiro:** Serra do Couto, s.d., *A. Glaziou 2153* (RB-30604 [on 2 sheets]); Serra do Couto, s.d., *A. Glaziou 3450* (CESJ). **São Paulo:** Salesópolis, Boracéia, 4 Mar 1962, *O. Travassos 336* (RB-on 2 sheets); Serra de Paranapiacaba, Sep 1925, *A.C. Brade 8402* (MBM, RB-on 2 sheets).

Oleandra brasiliana is the most widespread species in the Brazilian Atlantic Forest, occurring from the state of Bahia southwards to São Paulo (Fig. 3). A disjunct population in the state of Ceará is in the Baturité region, which is an "island" of wet forest within the Brazilian semi-arid vegetation (*Caatinga* biome), presenting many species in common with the Atlantic Forest (Brade, 1940; Freitas and Matias, 2010:Fig. 1).

Oleandra brasiliana has glabrescent laminae: *i.e.*, hairs are found in juvenile fronds, but they fall off in older ones. Most specimens seen appear to have fully glabrous laminae (Figs. 1H–J), but a detailed examination reveals scattered catenate and glandular hairs on both sides of the midribs, adaxially on the laminar tissue between the veins, and on the laminar margins (especially on the laminar apices). The other Brazilian Atlantic Forest species all have pubescent laminae (each with a different pattern of indument), and the laminae remain pubescent as they age.

Among the Brazilian Atlantic Forest species, *Oleandra brasiliana* is the most similar to *O. articulata*. *Oleandra brasiliana* differs from *O. articulata* by glabrescent midribs, with scattered catenate and glandular hairs, lacking any kind of scales, whereas in *O. articulata* the midribs present conspicuous cordate reddish scales abaxially, and lack any kind of hairs. *Oleandra articulata* is also glabrous throughout the laminae, while *O. brasiliana* is glabrescent, with scattered catenate and glandular hairs especially found on midribs, laminar tissue between the veins (adaxially), and laminar margins.

Oleandra hirta Brack., U.S. Expl. Exped., Filic. 16:214, t. 29. 1854. *Oleandra neriiformis* Cav. var. *hirta* (Brack.) Baker, Fl. Bras. [Martius] 1(2):495. 1870. LECTOTYPE (designated by Maxon, 1914:394).—BRAZIL. **Rio de Janeiro:** Organ Mountains, near Rio de Janeiro, 1838–1842, *Herb. U.S. South Pacific Expl. Exped, Capt. Wilkes s.n.* (US-00810702, image seen; isolectotype: K-000590573, image seen). Figs. 1K, L, 3.

Plants epiphytic or rupestrial. *Rhizomes* dark brown, pruinose, ca. 2 mm diam.; *scales* not seen. *Phyllopodia* 25–42 mm long. *Fronds* 50–55 cm long; *petioles* 2.5–4 cm long, brown; *laminae* linear, long-attenuate at base, acuminate to slightly aristate at apex, 45–50 × 3–3.2 cm; *midribs* abaxially and adaxially with 3–5-celled hairs (catenate and/or glandular?) and fimbriate scales; *veins* partly anastomosing, with hairs on both sides; *laminar tissue* *between the veins* with hairs on both sides; *laminar margins* with hairs; *sori* infra-medial; *indusia* reniform, dark brown, with hairs; *spores* not seen.

Distribution and ecology.—Endemic to the state of Rio de Janeiro; elevation unknown (probably ca. 1,000–1,500 m) (Fig. 3).

Oleandra hirta is known only from the type collection, which was made by the United States Exploring Expedition in the mid 19th century. The linear laminae (Fig. 1K) make *Oleandra hirta* more similar to Old World species, such as *O. neriiformis* Cav. and *O. mollis* C.Presl, and the partly anastomosing veins (Fig. 1L) are unique in the genus. Further collections are needed to better understand this species and to propose conservation actions.

Oleandra quartziticola Schwartsb. & J.Prado, ***sp. nov.*** Type.—BRAZIL. **Espírito Santo:** Vargem Alta, Morro Branco, 20°39′28"S, 41°00′18"W, 640 m, 17 Sep 2015, *P.B. Schwartsburd & C.V. Miranda 3522* (holotype: SP!; isotypes: FI!, NY!, RB!, UC!, UPCB!, VIC!-on 2 sheets). Figs. 2G–J, 3.

Rhizome scales bicolorous; laminae elliptical; midribs, veins, and laminar margins with catenate and glandular hairs; laminar tissue between the veins abaxially glabrous, adaxially with catenate and glandular hairs.

Plants rupestrial. *Rhizomes* light brown, not pruinose, 1–3.5 mm diam.; *scales* bicolorous, centrally black, orange at the margins, densely glandular-ciliate. *Phyllopodia* 15–20 mm long. *Fronds* 18–30 cm long; *petioles* (2–)3–5.5 cm long, stramineous to brown; *laminae* elliptical, cuneate at base, acuminate or aristate at apex, 15–25 × 2–4.2 cm; *midribs* with 4–7-celled catenate hairs and 4–7-celled glandular hairs on both sides; *veins* free, with catenate and glandular hairs on both sides; *laminar tissue between the veins* abaxially glabrous, adaxially with sparse catenate and glandular hairs; *laminar margins* with glandular hairs and a few catenate hairs; *sori* infra-medial; *indusia* orbicular-reniform, bicolorous, centrally dark brown, light brown at the margins, glabrous; *spores* light brown.

Distribution and ecology.—Endemic to the state of Espírito Santo; ca. 600–700 m elev. (Fig. 3).

Etymology.—The specific epithet refers to the habit of the species, which grows on quartz boulders. *Quartz* (= quartz) + *ito* (= diminutive form used as "stone of") + *cola* (growing on).

Specimens Examined.—BRAZIL. **Espírito Santo:** Vargem Alta, ES-164, no km 332, 20°39′26.7"S, 41°00′20.0"W, 663 m, 23 Jan 2011, *J.R. Pirani et al. 6209* (SP, SPF); Vargem Alta, Morro Branco, 20°39′26"S, 41°00′19"W, 660 m, 17 Sep 2015, *P.B. Schwartsburd & C.V. Miranda 3525* (SP, VIC).

Oleandra quartziticola is endemic to the state of Espírito Santo, and it is characterized by bicolorous rhizome scales, elliptical laminae, catenate and glandular hairs present on both sides of the midribs, veins, and laminar margins, glabrous laminar tissue between the veins (abaxially), and bicolorous indusia (Figs. 2G–J). Besides having different geographical ranges, *O. quartziticola* differs from *O. baetae* by longer fronds, 18–30 cm long (*vs.* 9–15(–22) cm), longer petioles, (2–)3–5.5 cm long (*vs.* 3–5 mm), laminae proximally cuneate (*vs.* truncate or inequilateral), and laminar tissue between the veins abaxially glabrous (*vs.* with catenate and glandular hairs) (Figs. 2G–J *vs.* 1A–D).

HYBRIDS

Oleandra baetae × ***Oleandra brasiliana*** Fig. 2A, B.

Plants rupestrial. *Rhizomes* dark brown, pruinose, 2–4 mm diam.; *scales* bicolorous at base (darker only at the attachment point, orange in the remaining parts), moderately glandular-ciliate. *Phyllopodia* 0.5–4.5 cm long. *Fronds* 13–25(–30) cm long; *petioles* 0.5–4 cm long, stramineous; *laminae* obovate, cuneate at base, acuminate to aristate at apex, 12–22 × 2.5–5.5 cm; *midribs* with 6–10-celled catenate hairs and 3–5-celled glandular hairs on both sides; *veins* free, with catenate and glandular hairs on both sides; *laminar tissue between the veins* with catenate and glandular hairs on both sides, or rarely glabrescent; *laminae margins* with catenate and glandular hairs; *sori* few, infra-medial; *indusia* orbicular-reniform, light brown, with sparse glandular hairs or glabrescent; *spores* mostly aborted, a few well-formed and reddish-brown.

Distribution and ecology.—Endemic to the state of Minas Gerais, from places where the putative parents occur sympatrically; ca. 900–1,500 m elev.

SPECIMENS EXAMINED.—BRAZIL. **Minas Gerais:** Lima Duarte, Nascentes do Pirapitinga, ca. 1,000 m, 11 Oct 1989, *L. Krieger & M. Brugger 24240* (CESJ, VIC-47051); Lima Duarte, Serra Negra, 21°55′49.9"S, 43°46′43.1"W, 986 m, 15 Nov 2008, *F.R.G. Salimena & P.H. Nobre 2757* (MBM, UPCB, RB); Mariana, Córrego João Manoel Samarco, 8 Nov 1989, *M.A. Zurlo & J.L. Silva 2363* (OUPR); Ouro Preto, Cachoeira das Andorinhas, 1937, *J. Badini s.n.* (OUPR-10831); Ouro Preto, Cachoeira das Andorinhas, 1,200 m, 23 May 1979, *L. Mantone et al. 717* (RB); Rio Preto, margens do Rio do Funil, 22°00′S, 43°53′W, 930 m, 26 Feb 1999, *R. Mello-Silva et al. 1658* (RB, SP, SPF); Serra do Frasão, 1942, *J. Badini 4179* (OUPR); Locality unknown, Dec 1968, *Anonymous s.n.* (OUPR-5800).

This putative hybrid involving *Oleandra baetae* and *O. brasiliana* is known from the few places where both putative parents occur sympatrically: in the mountains of Serra do Frasão, Serra Negra, and Serra de Ouro Preto, Minas Gerais (see Fig. 3). It presents intermediate morphology between the two putative parents: the pruinose rhizomes, the rhizome scales bicolorous at base, and the laminar indument (with catenate and glandular hairs throughout) are similar to *O. baetae* (Figs. 1B–D, 2B); whereas the long phyllopodia (0.5–4.5 cm long) and petioles (0.5–4 cm long), and the laminar architecture (obovate, cuneate at bases, aristate at apices) are similar to *O. brasiliana* (Figs. 1E, F, 2A). In addition, this putative hybrid produces sparse sori, and most spores are aborted (only a few are well formed).

Dubious Records

BRAZIL. **Santa Catarina.**—Christ (1900: 29) cited a specimen of "*Oleandra nodosa*" (= *O. articulata*) from "Rochers des bords du Pirahy mirim", s.d., *W. Schwacke* (P?). We have not seen this material, nor any *Oleandra* specimen from the state of Santa Catarina, and Sehnem (1979) does not list any *Oleandra* from there. The southernmost record of *Oleandra* in South America seen by us is that of *O. australis*. Possibly, the material cited by Christ (1900) represents *O. australis* or, less likely, *O. brasiliana*.

Acknowledgments

We thank the following curators and herbarium staff by their assistance during our visits: Maria Cristina T. B. Messias, Viviane R. Scalon, and Ana Paula Fortuna-Pérez (OUPR), Paulo H. Labiak, Renato Goldenberg, and Laura C. de Lannoy (UPCB), Rafaela Forzza and Claudine Mynssen (RB), José T. W. Motta (MBM), and Vinícius A. O. Dittrich (CESJ). We also thank Reinaldo Pinto for making the drawings, João A. P. Araújo for helping in the field expedition in Ceará, Raquel Santana-Milagres for helping in the field expedition in Minas Gerais and general support, Robbin Moran for sending images from NY, and the handling editor (Carl Rothfels) and the two anonymous reviewers for their critical suggestions on the early version of this paper.

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American Fern Journal 106(3):206–222 (2016)
Published on 19 October 2016

Systematics of *Diplazium laffanianum* (Athyriaceae), a Fern Species Endemic to Bermuda

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Abstract.—*Diplazium laffanianum* was last collected from its natural habitat in Bermuda in 1905. Now the species is considered extinct in the wild as it exists only in greenhouses, tissue cultures, and as a few young reintroduced sporophytes. For the last 13 years, a collaborative team of researchers from the Government of Bermuda and the Lab for Rare & Endangered Plants at Omaha's Henry Doorly Zoo in Nebraska, USA has been working to bring this species back from the brink of extinction. In this project, we developed propagation methods for *D. laffanianum*, and we studied molecular and morphological characters to determine the phylogenetic placement of *D. laffanianum*. We sequenced plastid DNA from *D. laffanianum* using high-throughput sequencing, downloaded six plastid markers (*atpA, atpB, matK, rbcL, rps+rps4-trnS,* and *trnL* intron+*trnL-F*) from GenBank for 21 *Diplazium* and outgroup taxa, and inferred a phylogeny. We examined 185 collections of five *Diplazium* species closely related to *D. laffanianum*, and we scored 15 morphological characters to test whether *D. laffanianum* is a distinct species. The phylogeny placed *D. laffanianum* in the *D. cristatum* group, suggesting a Neotropical ancestor, and two morphological characters distinguished *D. laffanianum* from close relatives. Our results provide evidence for the phylogenetic placement of *D. laffanianum*, and we are actively propagating and reintroducing individuals to the wild, aiding conservation of the species.

Key Words.—cultivation, extinction, molecular phylogeny, reintroduction, Woodsiaceae

Diplazium laffanianum (Baker) C. Chr. was first described by Mr. John G. Baker (1882) who placed it in the genus *Asplenium* L. As a botanist and curator at the Royal Botanic Gardens Kew, Baker received a living individual of a

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Bermudian fern from Lieutenant General Sir Robert Laffan, Governor of Bermuda in 1880 (Britton, 1918). Baker allowed the young plant to grow to reproductive age and then determined that it was a distinct species, closely related to other *Asplenium* species in the West Indies such as *Asplenium crenulatum* (Liebm.) Baker, now called *Diplazium striatum* (L.) C. Presl (Baker, 1882). *Diplazium laffanianum* was moved from *Asplenium* to *Diplazium* Sw. by Christensen (1905). Sir Robert Laffan was honored in both the scientific name and the common name, "Governor Laffan's Fern".

Dr. N.L. Britton (1918) reported that *Diplazium laffanianum* was found in Bermuda in cave mouths and rock crevices in the early 1900s between Harrington Sound and Paynter's Vale. Although he observed the species in its natural habitat in 1905, Britton (1918) was unable to find it in later years, and no one else has reported seeing it in the wild since that time. In 1914, from two plants housed in a private greenhouse in Hamilton, Bermuda, Britton (1918) attempted to raise plants from spores, with no success.

Beginning in the 1960s, the last few remaining individuals of *Diplazium laffanianum* (obtained from Mrs. William E. S. Zuill, an important conservation visionary who cared for the ferns for over 30 years) were housed at the Bermuda Botanical Gardens (Jones, 1963). These individuals remained at the Gardens until September of 2001, when all five ferns were moved to the Government Nursery at Tulo Valley. In September 2003, hurricane Fabian hit Bermuda, destroying the greenhouse at Tulo Valley, killing two ferns and damaging the remaining three. In 2010, only one mature fern remained (Sarkis, 2010), and it has since died. Serendipitously, just prior to hurricane Fabian, in late 2003, two sporulating fronds of *D. laffanianum* were sent to the Lab for Rare & Endangered Plants at Omaha's Henry Doorly Zoo (OHDZ) in Omaha, Nebraska, USA, with hopes that the plant conservation experts could begin a propagation and reintroduction program for the species. These two, 4 cm fronds, each from a different sporophyte provided the only reproductive germplasm available for propagation. Since that time, authors MF and ML developed protocols for *in vitro* micropropagation, which produced thousands of juvenile plants. Several individuals were subsequently acclimatized *ex vitro* and sent to Bermuda, where author AC is reintroducing them at two of the historical sites. In 2014, *D. laffanianum* was listed as "Extinct in the Wild" by the IUCN Red List of Threatened Species (www.iucnredlist.org/details/56605187/0). Because it is endemic to the small island of Bermuda where it is restricted to seaside cave mouths, few specimens of *D. laffanianum* have been collected for herbaria, and the species has never been included in a phylogeny of the genus (Wei, Schneider, and Zhang, 2013; Wei *et al.*, 2015). A well-supported phylogenetic placement will aid conservation efforts of this rare species by revealing its distinction from closely related species, documenting its evolutionary history, and lending its weight to biodiversity assessments (Vázquez and Gittleman, 1998; Steele and Pires, 2011; Forest *et al.*, 2015; Tucker *et al.*, 2016).

Diplazium s.l. is a monophyletic genus of ferns containing 350–400 species (Christensen, 1906; Kato, 1977; Kuo *et al.*, 2011; Wei, Schneider, and Zhang, 2013), with *D. plantaginifolium* (L.) Urb. as the type species. Familial placement of the genus has fluctuated historically. Previously, *Diplazium* was placed in Woodsiaceae Herter (Swartz, 1801; Smith *et al.*, 2006), but this family was found to be paraphyletic (Sano *et al.*, 2000; Schuettpelz and Pryer, 2007; Rothfels *et al.*, 2012b). More recently, *Diplazium* was placed in Athyriaceae Alston in the eupolypod II clade of ferns (Rothfels *et al.*, 2012a). To date, there has not been a complete infrageneric classification of *Diplazium* (Tryon and Tryon, 1982).

The pantropical genus *Diplazium* is estimated to have originated in Eurasia and then migrated through the boreotropics to tropical America (Wei *et al.*, 2015). Species richness is primarily centered in Malesia (ca. 70%) and the Neotropics (ca. 25%; Tryon and Tryon, 1982; Pacheco and Moran, 1999; Mickel and Smith, 2004), with the remainder (< 5%) found in Australia (Jones, 1998) and the Afro-Madagascan region (Roux, 2009). Before this study, morphological characters distinguishing *Diplazium laffanianum* had not been published, and its nearest relatives had not been identified. When Baker (1882) described *D. laffanianum*, he distinguished the species from *D. striatum*, which turns out to be quite distantly related (see below).

In this study, propagation and reintroduction methods were developed for *Diplazium laffanianum*. Additionally, molecular and morphological data were used to investigate the following questions:

1) What is the phylogenetic placement of *D. laffanianum*?
2) What morphological characteristics distinguish *D. laffanianum* from other species of *Diplazium*?
3) From which geographical region did *D. laffanianum* originate?

MATERIALS AND METHODS

Propagation.—At OHDZ, two small spore samples from the two specimens of *Diplazium laffanianum* received from Bermuda (taken from the Tulo Valley individuals vouchered at the Royal Botanic Gardens, Kew [Pembroke Parish, Tulo Valley Nursery, 23 February 2007, *M.A. Hamilton HS615*]) were surface sterilized with 10% household bleach (5.25% NaOCl v/v), rinsed three times with sterile, distilled water, and cultured on Murashige and Skoog (MS) agar-gelled medium (Murashige and Skoog, 1962) in 100 × 20 mm petri dishes (Phytotechnology Labs, Overland Park, KS, USA). After adding 0.6% agar (w/v) and 20 g sucrose, the pH was adjusted to 5.7, the medium was autoclaved in flasks capped with three layers of aluminum foil at 121° C and 1.4 kg/cm^2 for 15 min, and then it was poured into petri dishes under a laminar flow hood and cooled until gelled. No plant growth regulators were added to the agar medium. Cultures were incubated under room temperature regime (25 + 3°C) with a 16 hr photoperiod under cool white fluorescent illumination (100 / μmol*m^2*s PFD). Spores cultured in petri dishes commenced germinating after 15 days, and observations were made weekly. Development of prothalli was observed and recorded using a Leica EZ4W stereo microscope (Visualab, G.W. Brown & Co., Waterloo, NE, USA) and photographed with a Canon Power Shot camera at 8x optical zoom (Fig. 1). After two months, individual young sporophytes were transferred to a soil mix of 3:1 Promix:sand, covered with plastic domes to maintain humidity, and placed under 60% shade cloth in the greenhouse for further development.

A first attempt to transplant ferns raised at OHDZ into the wild was carried out in the autumn of 2014. As there is almost no historical literature describing the habitat in which *Diplazium laffanianum* was found, the reintroduction was (and is still being) conducted as a trial and error approach over a variety of microhabitats. Between 24 November 2014 and 4 March 2015, 41 young *D. laffanianum* sporophytes were planted at three sites in the Walsingham Nature Reserve (Fig. 2). Additionally, in January 2015, eight ferns were placed in the Bermuda Audubon Society's nature reserve at Sear's Cave. Sear's Cave lies outside the known historic range of the species, but the habitat is similar to known sites and Sear's Cave already hosts populations of other rare ferns. Approximately 42 small patches of prothalli (gametophytes) were also placed across the four sites. Relative humidity, temperature, and light sensors were placed at each planting site.

Molecular.—Diplazium laffanianum prothalli were collected from the *in vitro*-germinated spores harvested from fronds taken from the Tulo Valley individuals vouchered at the Royal Botanic Gardens, Kew (Pembroke Parish, Tulo Valley Nursery, 23 February 2007, *M.A. Hamilton HS615*). Tissue was dried over silica for one week. Total genomic DNA was extracted from the tissue using the IBI Mini Genomic DNA Kit (IBI Scientific, Peosta, Iowa, USA) per the manufacturer's instructions. Approximately 8 μg of DNA (pooled from multiple gametophytes) was sent to the University of Missouri DNA Core Facilities for library preparation, and sequencing on the Illumina Hi-Seq platform at 12 samples per lane with single-pass, 100-bp reads (i.e., 1/12 of one lane was allocated for *D. laffanianum*).

The resulting sequences (9.4 million reads) were assembled using reference-based mapping to a complete plastid genome (*Pteridium aquilinum* subsp. *aquilinum*; GenBank accession number NC_014348; Der *et al.*, *unpublished*) in Geneious 6.1.7 (Biomatters, Inc., www.geneious.com). From this assembly, we selected six markers (*atpA, atpB, matK, rbcL, rps4+rps4-trnS* intergenic spacer (IGS), and *trnL* intron+*trnL-F* IGS) that had been used in the most recent phylogeny of *Diplazium* (Wei, Schneider, and Zhang, 2013), and we concatenated them into a single sequence. This sequence was aligned with equivalent concatenations from 17 other *Diplazium* species and two *Athyrium* species downloaded from GenBank (www.ncbi.nlm.nih.gov/genbank/; Appendix 1). This taxon sample was selected by comparing our preliminary results with the most complete phylogeny of *Diplazium* to date (Wei, Schneider, and Zhang, 2013). *Diplazium laffanianum* falls into major clade II; therefore, we included all members of major clades I, II, III, plus four representatives (*D. striatum* plus three species selected at random) from clade IV (Wei, Schneider, and Zhang, 2013). There are other *Diplazium* species in the West Indies that may be closely related to *Diplazium laffanianum*, such as

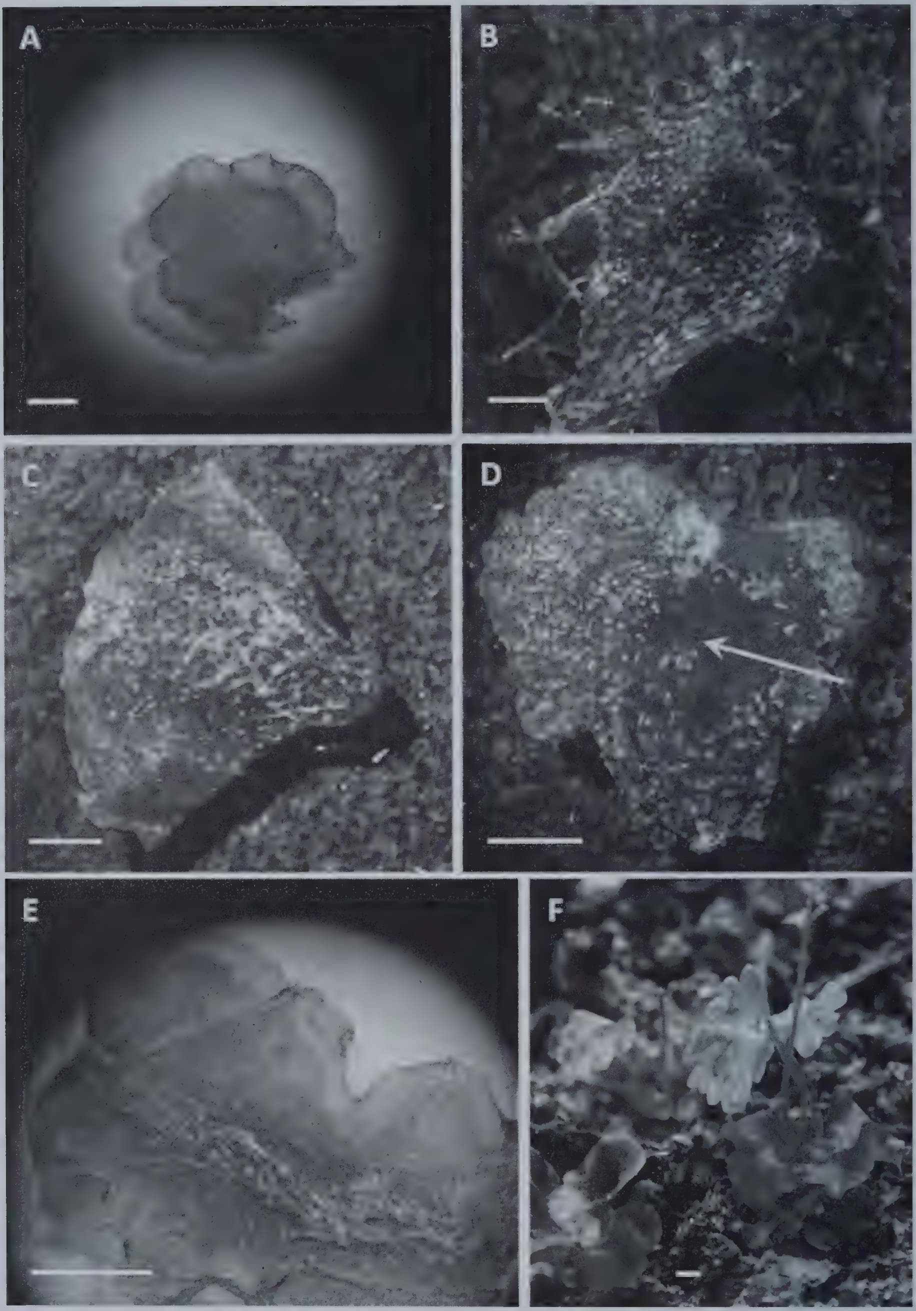

Fig. 1. Stages of *Diplazium laffanianum* gametophyte development from spores cultured *in vitro* (days after germination in parentheses). (A) Early stage gametophyte (25 days), (B) Filamentous prothallus (25 days), (C) Ameristic male prothallus with hair-like rhizoids (25 days), (D) Cordate prothallus with rhizoids and arrow indicating archegonia (30 days), (E) Elongated prothallus with antheridia and rhizoids (45 days), and (F) Early sporophyte with fronds (45 days). Scale bar = 1 mm

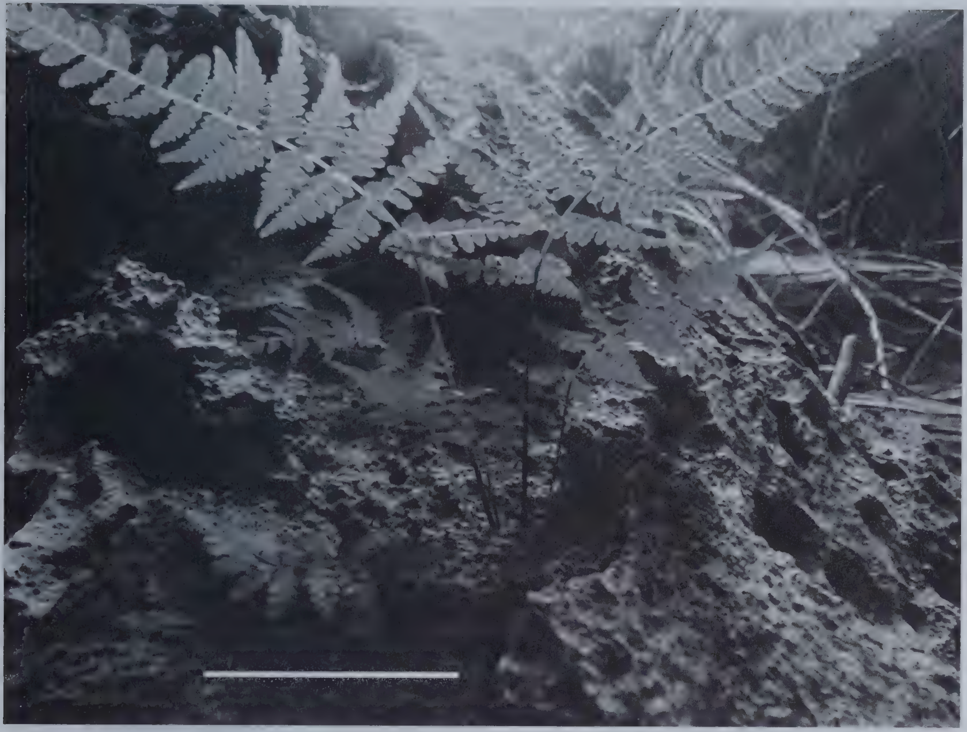

FIG. 2. Cultivated *Diplazium laffanianum* restored to Zuill's Cave, Bermuda in January 2015. Photograph taken 9 November 2015 (approximately nine months after reintroduction). Scale bar = 5 cm.

D. mildei (Kuhn) C.Chr., *D. lonchophyllum* Kunze, *D. drepanolobium* A.R.Sm., and *D. stuebelianum* (Hieron.) Stolze (Stolze, Pacheco, and Øllgaard, 1994; Mickel and Smith, 2004); however, these species were not included in Wei, Schneider, and Zhang (2013), and no GenBank data exist for these taxa except the *rbcL* gene for *D. lonchophyllum*. *Diplazium lonchophyllum* is the *Diplazium* species that looks most similar to *D. cristatum* (Proctor, 1985); therefore, we included this sequence in our alignment, with the other regions in the concatenation coded as missing data. Sequences were aligned in Geneious using the MAFFT plug-in (Katoh *et al.*, 2002).

Maximum likelihood (ML) phylogenies were estimated using RAxML, version 8 (Stamatakis, 2014) accessed through the online CIPRES Science Gateway (www.phylo.org). The analysis was partitioned into six loci and conducted using the best fit model of evolution (GTR + I + Γ) according to the Akaike information criterion (AIC; Akaike, 1974) implemented in jModelTest

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(A–F). All stages recorded using a Leica EZ4W stereo microscope (Visualab, G.W. Brown & Co., Waterloo, NE, USA) and photographed with a Canon Power Shot camera at 8x optical zoom.

TABLE 1. Morphological characters observed and measured on *Diplazium laffanianum* and five close relatives.

Species	Lamina	Lamina shape	Pinnae margins[a]
D. laffanianum	1-pinnate	deltoid	pinnatifid to deeply divided
D. bombonasae	1-pinnate	oblong-lanceolate	entire to crenate
D. cristatum	1-pinnate	lanceolate	nearly entire to shallowly lobed
D. lonchophyllum[c]	1-pinnate	lanceolate	shallowly lobed to pinnatifid
D. plantaginifolium	simple, entire	lanceolate-elliptic	N/A[d]
D. unilobum[b]	1-pinnate	lanceolate	entire to crenate

Note: N/A indicates the character is not present for measurement or observation; "–" indicates the character was not measured.

[a] Characters used to distinguish *Diplazium laffanianum* from other species in the study.

[b] Lamina is divided into pinnae; therefore, the base is described as "Pinnae basal lobe shapes".

[c] specimens observed in herbarium images.

[d] Lamina is not divided into pinnae.

(Posada, 2008). Trees were rooted using *Athyrium filix-femina* (L.) Roth and *Athyrium otophorum* (Miq.) Koidz. because *Athyrium* and allies (the "athyriids") are the sister group to *Diplazium* (Rothfels *et al.*, 2012b; Wei, Schneider, and Zhang, 2013). ML analyses utilized the automated stopping criterion, terminating a search when the likelihood score remained constant for 20,000 consecutive generations. Likelihood scores of the optimal trees generated by RAxML were calculated in PAUP* (Swofford, 2003), which better optimizes branch lengths. ML bootstrap analyses were performed in RAxML on 1000 replicates using an automated stopping criterion set at 20,000 generations.

Morphological.—Following the results of the phylogenetic analyses, collections of the five *Diplazium* species occurring in the clade with *Diplazium laffanianum* were observed in herbarium images on the Internet or were borrowed from the following herbaria: F, K, MO, NEB, and NY. Eight morphological characters were described, and seven were measured (Table 1) on multiple representatives of each species as follows (numbers in parentheses indicate quantities; "*" indicates that herbarium images were examined): (30) *D. bombonasae*; (68) *D. cristatum*; (10) *D. laffanianum*; (5*) *D. lonchophyllum*;

TABLE 1. Extended.

Pinnae basal lobe shapes[a]	Pinnae apex	Lamina base	Frond surface (top and bottom)	Venation
primarily equal (some slightly inequilateral)	acute-acuminate	N/A[b]	glabrous	reticulate
extended basal lobe on the acroscopic side	acute-acuminate	N/A[b]	glabrous	reticulate
inequilateral with acroscopic side larger	acute-acuminate	N/A[b]	glabrate	reticulate
inequilateral (some not strongly) with acroscopic side larger	acute-acuminate	N/A[b]	–	–
N/A[d]	acute-acuminate	0–3 lobes at base	glabrous	reticulate
extended basal lobe on the acroscopic side	acute-slightly rounded	N/A[b]	–	–

(67) *D. plantaginifolium*; and (3*) *D. unilobum* (Poir.) Hieron. (Representative examples are listed in Appendix 2).

RESULTS

Propagation.—Fern cultures were successfully initiated, and high numbers of immature gametophytes were produced from both initial spore samples. *Diplazium laffanianum* spores are <50 μm long and spore densities *in vitro* were variable across petri dishes (100–600 spores per petri dish). As gametophytes developed, they were re-cultured on fresh MS medium in larger culture vessels (Magenta Corp., Bio-world, Dublin, OH) where they sporadically produced sporophyte structures after two months. The fern prothalli produced many hyaline rhizoids, but many were slow to produce robust rhizoids for sustained growth. Survival of the largest sporophytes transferred to the potting soil mix was low (ca. 5%) in early tests. Survival was later improved to around 95% by adding native soil collected from the Walsingham Nature Reserve to the potting mix and keeping potted ferns in closed tanks to maintain high humidity.

Initial survivorship of sporophytes has varied across the four locations. The plantings at Zuill's Cave survived well, and as of September 2015, six large sporophytes have survived (Fig 2). The November 2014 plantings at a second site in the Walsingham Reserve all died within three weeks.

Molecular.—The six plastid regions (*atpA, atpB, matK, rbcL, rps4+rps4-trnS* IGS, and *trnL* intron+*trnL-F* IGS) for *Diplazium laffanianum* were sequenced

TABLE 1. Extended.

Frond length (cm)	Pinnae length (mm)	Width of smallest pinna (mm)	Width of largest pinna (mm)	# of pinnae[a]	# of sori per pinna	Sorus length (mm)
26–37	15–61	3–4	10–23	(12)26–28	10–14	1–4
26–79	21–69	2–6	9–25	22–51	11–38	2–6
21–107	35–170	3–20	12–61	16–40	6–120	2–9
–	–	–	–	23–35	–	–
23–60	N/A	N/A	N/A	N/A	32–175	8–20
–	–	–	–	13–38	–	–

with adequate (> 30x) coverage (according to Straub *et al.*, 2012) and deposited in GenBank (marker lengths and partition ranges shown in Table 2). The phylogeny (alignment available in TreeBase, accession #19304) had moderate (>75) to high (>90) bootstrap (BS) scores for most clades (Fig. 3). *Diplazium laffanianum* was nested within the *D. cristatum* group, and evolutionary differences (i.e., branch lengths) are evident between *D. laffanianum* and the closest relatives in our study—*D. lonchophyllum, D. cristatum,* and *D. unilobum.*

Morphological.—The measured morphological characters showed great overlap among *Diplazium laffanianum* and the closest relatives examined in

TABLE 2. Illumina sequence coverage, GenBank accession numbers, length, and data partitions for six plastid regions in *Diplazium laffanianum.*

Plastid DNA region	Average coverage	GenBank Accession #	Length (bp[a])	Data partitions (bp range)[b]
atpA	201 x	KU363748	1524	1-1775
atpB	216 x	KU363749	1482	1776-3061
matK	242 x	KU363750	1403	3062-3860
rbcL	226 x	KU363751	1428	3861-5168
rps4+rps4-trnS IGS	264 x	KU363752	351	5169-6076
trnL intron+*trnL-F* IGS	235 x	KU363753	794	6077-6922

[a] bp = nucleotide base pairs.

[b] aligned lengths including gaps.

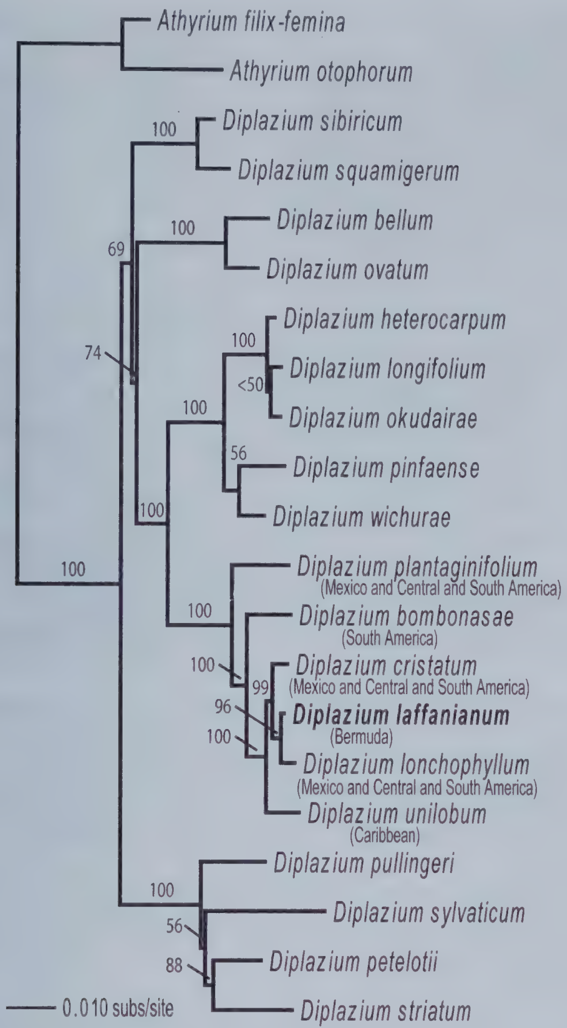

FIG. 3. Maximum likelihood (ML) phylogeny (-ln L = 20011.47) for 19 species of *Diplazium* plus two *Athyrium* species as outgroups, estimated from six concatenated plastid markers (*atpA*, *atpB*, *matK*, *rbcL*, *rps4*+*rps4-trnS* IGS, and *trnL* intron+*trnL-F* IGS). Total aligned length = 6,922 bp. Numbers above branches indicate ML bootstrap support values resulting from 1000 replicates.

this study (Table 1). The two characters that best distinguished *D. laffanianum* were pinnae margins and pinnae basal lobe shapes (Fig. 4).

DISCUSSION AND CONCLUSIONS

Our investigation identified some of *Diplazium laffanianum*'s closest relatives and illuminated its distinctive morphological characters, but this Bermudian endemic must rely on its evolutionary adaptations to survive in the wild. Morphologically, *Diplazium laffanianum* is quite similar to *D.*

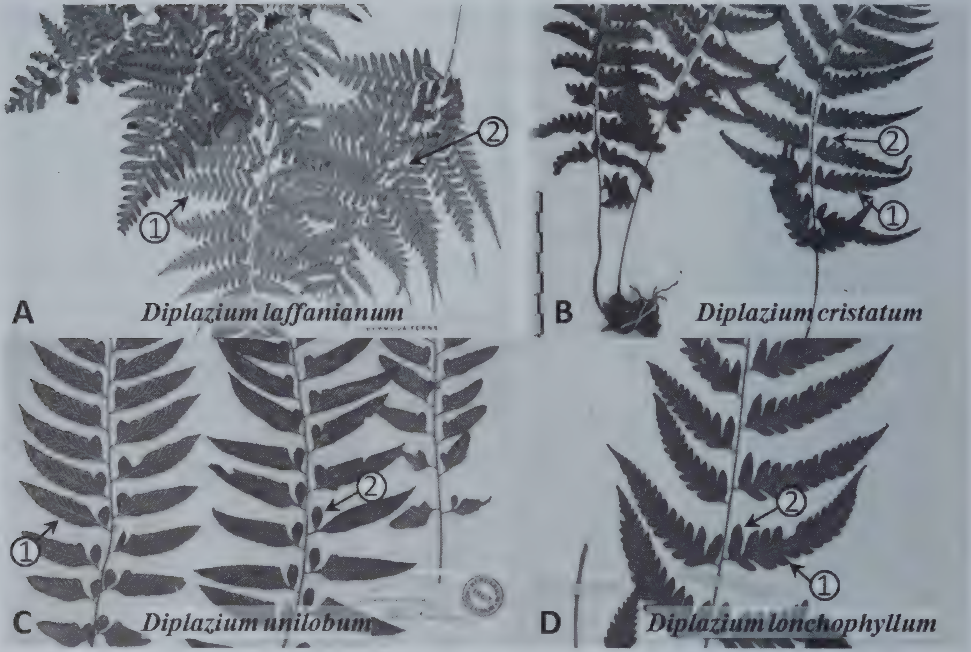

FIG. 4. Two morphological characters that distinguish *Diplazium laffanianum* (DLAF; A) from some of its closest relatives—*D. cristatum* (DCRS; B), *D. unilobum* (DUNI; C), and *D. lonchophyllum* (DLON; D): 1) Pinnae margins (pinnatifid or divided in DLAF; entire to shallowly lobed in DCRS and DUNI and lobed to infrequently pinnatifid in DLON), and 2) Pinnae basal lobe shapes (equal on the acroscopic side in DLAF; inequilateral on the acroscopic side in DCRS, DUNI, and DLON). Photos of herbarium vouchers as follows: (A) Bermuda. Church Cave, 5 July 1900, *Howe s.n.* (NY); (B) El Salvador. Ahuachapán, 5 March 1994, *Chinchilla ISB00151* (MO); and images accessed on the Internet as follows: (C) Jamaica. 1867, *Macfayden s.n.* (K*) (http://apps.kew.org/herbcat/getImage.do?imageBarcode=K000632788, accessed 22 March 2016); (D) Mexico, Veracruz, 1829, *Schiede s.n.* (B) (http://herbarium.bgbm.org/object/B200047289, accessed 22 March 2016).

lonchophyllum and *D. cristatum*; however, two characters are consistently different (Fig. 4; Table 1). The margins of almost all the lower pinnae in *D. laffanianum* are deeply divided (or pinnatifid), whereas the pinnae margins in *D. cristatum* and *D. lonchophyllum* are primarily crenate or shallowly lobed, or are pinnatifid only at the base (marked "1" in Table 1). The pinnae margins of the next closest relative, *D. unilobum*, are crenate to nearly entire above the one basal lobe. Additionally, the lobes at the bases of the pinnae vary among the species. In *D. cristatum* and *D. lonchophyllum*, the basal lobes are unequal, with the lobe on the acroscopic side being larger, and *D. unilobum* has only one basal lobe, and it is on the acroscopic side (marked "2" in Table 1). In *D. laffanianum*, the basal lobes are primarily equal, with only a few being slightly unequal.

When it was originally described, *Diplazium laffanianum* was reported to be closely related to *D. striatum* (Baker, 1882). However, our results indicated that

D. laffanianum is, instead, a member of the *D. cristatum* group within major clade II (Wei, Schneider, and Zhang, 2013). *Diplazium laffanianum* is most closely related to *D. lonchophyllum* (Fig. 3) and is phylogenetically distant from *D. striatum* (Fig. 3). The phylogenetic placement of *D. laffanianum* in the *D. cristatum* group, whose members are found in Mexico, Central and South America, and the Caribbean, suggests a Neotropical origin for the species. Since the Bermuda islands are volcanic in origin, dispersal of the Neotropical ancestor of *D. laffanianum* to Bermuda likely occurred via wind currents or through a major environmental event (such as a hurricane). The prevailing westerlies bring weather fronts from eastern North America across the islands, and tropical cyclones move up from the Caribbean in late summer and autumn. It is therefore not surprising that a number of species native to the West Indies and eastern North America are found in Bermuda's native flora. Although it lies at latitude 34° North, Bermuda remains frost-free due to the warming influence of the Gulf Stream, allowing the survival of a number of Caribbean species.

The Bermuda archipelago has relatively low terrestrial biodiversity due to its volcanic origin and mid-oceanic isolation (Thomas, 2004). The indigenous flora of the islands is composed of chance arrivals, i.e., species carried to the islands by winds, ocean currents, and migrating birds. Only the hardiest species have been able to thrive on Bermuda's poor soils and tolerate the salty winds and irregular rainfall. The site at Walsingham Reserve is a rockface, not an actively dripping cave mouth; therefore, the failure of the sporophytes reintroduced at that location was probably due to the lack of moisture. Ongoing data analyses from the relative humidity, temperature, and light sensors should help explain the survival success of plantings at certain sites over others.

With the goal of increasing sporophyte survivability *ex vitro*, further refinements of acclimatization techniques continue. Because the species was extirpated from the wild over 100 years ago, there are few historic records of the conditions under which the species once grew. Furthermore, invasive plants, particularly non-native trees, have altered many of the former sites (*pers. comm.* Dr. David Wingate, former Terrestrial Conservation Officer). Only repeated reintroduction experiments and long-term monitoring will reveal which locations are favorable for lasting survival. Bermuda typically experiences a dry period in April and May, followed by hot summer weather into October, which will challenge the planted ferns. Further introductions are planned for the cooler, wetter months from November to January.

Conclusions.—Diplazium laffanianum is a rare fern whose native habitat is limited to caves at the eastern end of Bermuda, and as a habitat-limited island endemic, the species has probably never been abundant. Conservation biologists are working to propagate and return this species to the wild—reintroduction and propagation studies continue at both the OHDZ laboratory and in Bermuda to develop additional methods of acclimatizing juvenile plants to an *ex vitro* state. With these ongoing studies, the profile of *D. laffanianum* has been raised internationally with its addition to the IUCN

Red List, and the fate of the species is looking brighter. However, further research of *D. laffanianum* is urgently required for survival of this highly endangered species. Confirmation of the endemic status of this species should ensure that it becomes a conservation priority and that current recovery action continues.

Acknowledgments

The authors thank the *AFJ* editors and anonymous reviewers for very helpful critical comments, the Fund for Undergraduate Scholarly Experiences (FUSE) awarded to DCH by the UNO Office of Research and Creative Activity, Dr. David Sutherland (OMA Herbarium curator), the MU Core Sequencing Facility, the following herbaria for access to voucher images: B, BR, FLAS, K, MO, P, and the following herbaria for specimen loans: F, K, MO, NEB, and NY.

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Appendix 1. GenBank accession numbers for six plastid regions (*atpA, atpB, matK, rbcL, rps4+rps4-trnS* IGS, and *trnL* intron+*trnL-F* IGS) for 18 *Diplazium* (*D*) and two *Athyrium* (*A*) species included in the sequence alignment used to estimate the phylogenetic placement of *Diplazium laffanianum.*

	Plastid DNA region					
Species	*atpA*	*atpB*	*matK*	*rbcL*	*rps4-trnS*	*trnL-F*
A. filix-femina	JF832096[a]	JF832154[a]	JF303941[b]	JF832056[a]	AF425152[c]	EU329076[d]
A. otophorum	JF832098[a]	EF463563[e]	JF832258[a]	EF463305[e]	JN168076[f]	AF515236[f]
D. bellum	KC254200[g]	KC254279[g]	KC254121[g]	KC254356[g]	KC254507[g]	KC254428[g]
D. bombonasae	JF832115[a]	EF463570[e]	JF832273[a]	EF463308[e]	–	–
D. cristatum	KC254206[g]	EF463572[e]	KC254127[g]	EF463310[e]	KC254515[g]	KC254434[g]
D. heterocarpum	KC254203[g]	KC254282[g]	KC254124[g]	KC254359[g]	KC254510[g]	KC254431[g]
D. lonchophyllum	–	–	–	DLU05920[h]	–	–
D. longifolium	KC254204[g]	KC254283[g]	KC254125[g]	KC254360[g]	KC254511[g]	KC254432[g]
D. okudairai	KC254201[g]	KC254280[g]	KC254122[g]	KC254357[g]	KC254508[g]	KC254429[g]
D. ovatum	KC254199[g]	KC254278[g]	KC254120[g]	KC254355[g]	KC254506[g]	KC254427[g]
D. petelotii	KC254262[g]	KC254341[g]	KC254183[g]	KC254414[g]	KC254570[g]	KC254492[g]
D. pinfaense	KC254202[g]	KC254281[g]	KC254123[g]	KC254358[g]	KC254509[g]	KC254430[g]
D. plantaginifolium	KC254205[g]	KC254284[g]	KC254126[g]	KC254361[g]	KC254514[g]	KC254433g
D. pullingeri	KC254209[g]	KC254288[g]	KC254130[g]	KC254365[g]	KC254516[g]	KC254437[g]
D. sibiricum	KC254208[g]	KC254287[g]	KC254129[g]	KC254364[g]	KC254513[g]	KC254436[g]
D. squamigerum	KC254207[g]	KC254286[g]	KC254128[g]	KC254363[g]	KC254512[g]	KC254435[g]
D. striatum	KC254242[g]	KC254321[g]	KC254162[g]	KC254395[g]	KC254548[g]	KC254470[g]
D. sylvaticum	KP318887[i]	KP318898[i]	KP318907[i]	KP318918[i]	KP318929[i]	KP318940[i]
D. unilobum	KP318893[i]	–	KP318913[i]	KP318924[i]	KP318934[i]	KP318946[i]
D. wichurae	EF463920[e]	EF463579[e]	JF832275[a]	AB042743[f]	JN168093[f]	AF515245[f]

Note: "–" indicates the region was not available for inclusion; additionally, portions of the following regions were missing: *D. bombonasae* (*atpA*); *D. wichurae* (Mett.) Diels (*atpA*); *D. striatum* (rbcL); *Athyrium filix-femina* (*trnL* intron+*trnL-F* IGS); and *Athyrium otophorum* (*rps4+rps4-trnS* IGS).

[a] Rothfels *et al.*, 2012.

[b] Kuo *et al.*, 2011.

[c] Smith and Cranfill, 2002.

[d] Adjie *et al.*, 2008.

[e] Schuettpelz and Pryer, 2007.

[f] Direct submission to GenBank, unpublished.

[g] Wei, Schneider, and Zhang, 2013.

[h] Wolf, Soltis, and Soltis, 1994.

[i] Wei *et al.*, 2015.

Appendix 2

Representative examples of the herbarium specimens whose morphological characters were measured or observed. Listed: Specimen collection site, collection date, collector and collector's number (herbarium).

Diplazium bombonasae

BOLIVIA. Province of Nor Yungas: 1400 m, 12 November 1987, *Solomon 17406* (MO!);

ECUADOR. Hacienda Cotapino (Concepcion): 500 m, 19–20 November 1968, *Harling 6945* (MO!); Taisha: 1500 ft, 15 February 1962, *Cazalet 7783* (NY!).

PERU. Amazonas Condorcanqui Province: 340 m, 28 June 1997, *Chavez 24269* (MO!); Rio Huasaga: 185 m, 13–16 November 1986, *Campos 11727* (MO!); Pongo de Manseriche: 300 m, 4 December 1931, *Mexia 6217* (MO!); Amazonas Bagua Province: 400 m, 25 May 1996, *R. Vasquez 20988* (NY!).

Diplazium cristatum

COSTA RICA. Alajuela: 1000–1200 m, 1 December 1988, *Herrera 2336* (MO!); Limon: 140–500 m, 1 August 1985, *Hammel 14317* (MO!).

DOMINICAN REPUBLIC. Province Santiago: 1200–1250 m, 4 March 1983, *Mickel 9173* (NY!); Province Barahona: 800 m, 24 February 1983, *Mickel 8998* (NY!); Province Barahona:800 m, 29 December 1982, *Zanoni 25178* (NY!).

HONDURAS. Department Olancho: 635 m, 7 February 1987, *Croat 64395* (MO!).

PERU. Cajamarca San Ignacio Province: 1250–1800m, 7 March 1997, *Campos 3507* (MO!)

Diplazium laffanianum

BERMUDA. Castle Harbour: 1957, Jones (Bermuda Botanical Gardens); Church Cave Tucker's Town: 31 August – 20 September 1905, *Brown 318* (NY!); Church Cave: 1900, *Howe s.n.*(NY!); Fernery of Mrs. William Black, Hamilton: 1900, *Howe s.n.* (NY!); Church Cave: 5 July 1900, *Howe s.n.* (NY!); Greenhouse of Mrs. Black: 22 May – 6 Jun 1914, *Brown 2213* (NY!); Church Cave: 5 July 1905, *Howe s.n.* (NY!); Cultivated in Bermuda Botanic Gardens: 1985, *Wallace 4* (K!); Pager: 1957, collector unknown (K!)

Diplazium lonchophyllum

BRAZIL. Mirador: 1932, *Galeotti 6289* (BR!, image seen). COLOMBIA. *Moritz s.n.* (P!, image seen).

COSTA RICA. Cartago: vicinity of Tres Rios, 1965, *Godfrey 67178* (FLAS!, image seen); Guanacaste, Rio Las Flores: 475–500 m, 24 January 1985, *Grayum 4859* (MO!, image seen).

MEXICO. Veracruz: 1829, *Schiede s.n.* (B!, image seen); Collection year unknown, *Leibold 27* (B!, image seen).

Diplazium plantaginifolium

BELIZE. Toledo: 250 m, May 1997, *Davidse 36213* (MO!).

BOLIVIA. La Paz, Franz Tamayo: 1212 m, 21 July 2010, *Cayola 4101* (MO!).

COSTA RICA. Province of Alajuela: 600 m, 8 February 1978, *Liesner 5201* (MO!).

DOMINICAN REPUBLIC. La Lanza: 900–1000 m, 27 February 1969, *Liogier 14308* (NY!); Province Barahona: 800 m, 24 February 1983, *Mickel 9033* (NY!).

NICARAGUA. Department of Rivas: 140–600 m, 18 January 1983, *Moreno 19656* (MO!).

PUERTO RICO. District of Humacao: 18 July 1966, *Stimson 3526* (NY!).

Diplazium unilobum

CUBA. 1859. *Wright 1032* (K!, image seen).

DOMINICAN REPUBLIC. San Rafael, Sierra de Neiba: 1700–2000 m, 6 August 1967, *Gastony 571* (MO!, image seen)

JAMAICA. 1867, *Macfayden s..n.* (K!, image seen).

American Fern Journal 106(3):223–226 (2016)
Published on 19 October 2016

SHORTER NOTES

A Case of Background Matching in the Caterpillars of *Xenotrachea* (Lepidoptera, Noctuidae) with the Fronds of *Polypodiodes amoena* (Polypodiaceae).—The degree of visual similarity between an animal and its habitat has long been considered a key determinant in the success of an animal's camouflage (Thayer, The Scientific Monthly, 7:481–494. 1918). A range of experiments has demonstrated the efficacy of background-matching to avoid predation as well as the mechanisms by which it is achieved (Stevenes and Cuthill, Pro. Biol. Sci. 273:2141–2147. 2006).

Background-matching is common in insects, for example, as reported for *Phylliidae* (Phasmatodea), *Tettigoniidae* (Orthoptera), and *Notodontidae* (Lepidoptera), where their wings or other body parts imitate the venation pattern, splotches, and even the damaged structures of living or dead angiosperm leaves (Wang *et al.*, PNAS 107:16212–16215. 2010). In addition, there are changes in larval coloration and body markings during the different developmental life stages of Lepidoptera. For example, the penultimate (fourth) instar of the swallowtail butterfly, *Papilioxuthus,* uses the tubercle structures on their surface to mimic bird droppings, whereas the fifth (last) instar has a camouflaging green color (Futahashi *et al.*, Dev. Genes Evol. 218:491–504. 2008).

Despite the long evolutionary history of ferns during which they developed a rich diversity of leaves, evidence for insect–fern mimesis has rarely been reported when compared with the large number of studies on insect-angiosperm interactions. Recent studies of insect–fern mimesis predicted that the larva or pupa of insects would have evolved to mimic the pinna or sori of ferns to avoid recognition by visually-oriented predators, such as birds or lizards (Barker *et al.,* Biotropica 37:314–316. 2004; Nervo *et al.,* Amer. Fern J. 101:317–318. 2011). Here we report a case of life stage-specific mimicry between Lepidoptera larvae and fern structures.

Polypodiodes amoena (Wall. ex. Mett) Ching (Fig.1A) is widely distributed in the south of China; most plants are found on rocks or at the bases of tree trunks. In September 2012, we conducted a field study on Qiyun Mountain, in the southwest region of Jiangxi, China. Here we found three herbivorous caterpillars on the damaged laminar tissue of *P. amoena*, each of a different size and with different color patterns. At first sight, the caterpillars were overlooked due to their visual resemblance to the pinna of *P. amoema.*

All of the caterpillars were green. The smallest one was about 2.3 cm in length, had 14 brown dorsal flecks ranging from 0.05 cm to 0.25 cm, with each fleck decorated with two black hairs (Fig. 1B). This structural pattern created visual similarity to a mature frond with sori. The other two larvae were 3.5 cm and 5.1 cm in length, respectively. They had a diamond-shaped marking with blackish-brown edges and a yellow center in the middle of their back, and two linear stripes on the dorsal surface of the tail. These markings camouflaged the

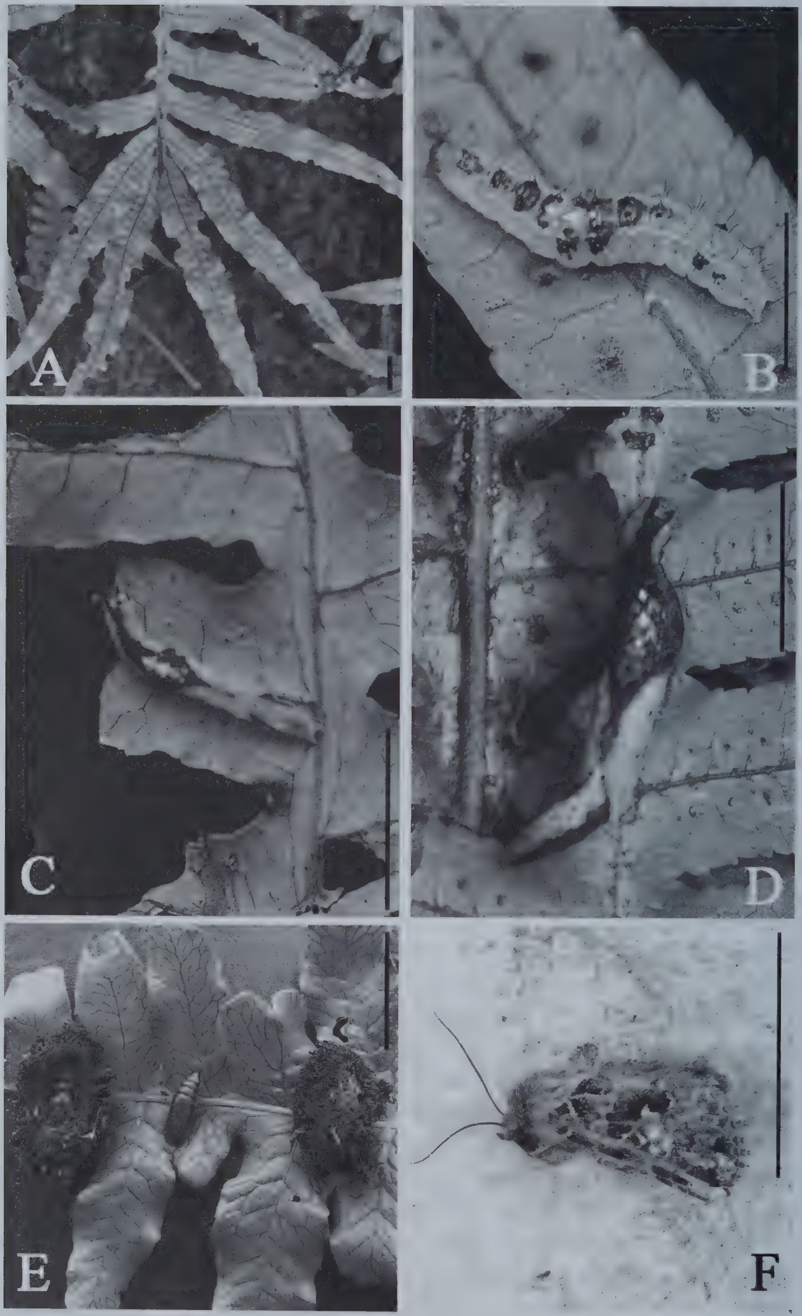

FIG. 1. *Xenotrachea* herbivory on *Polypodiodes amoena*. A. Part of the damaged frond of *Polypodiodes amoena*; B–D. Larvae of *Xenotrachea* sp. on damaged frond; E. Pupae of *Xenotrachea* sp.; F. Adult of *Xenotrachea* sp. Scale bars: A, C–F, 2 cm; B, 1 cm.

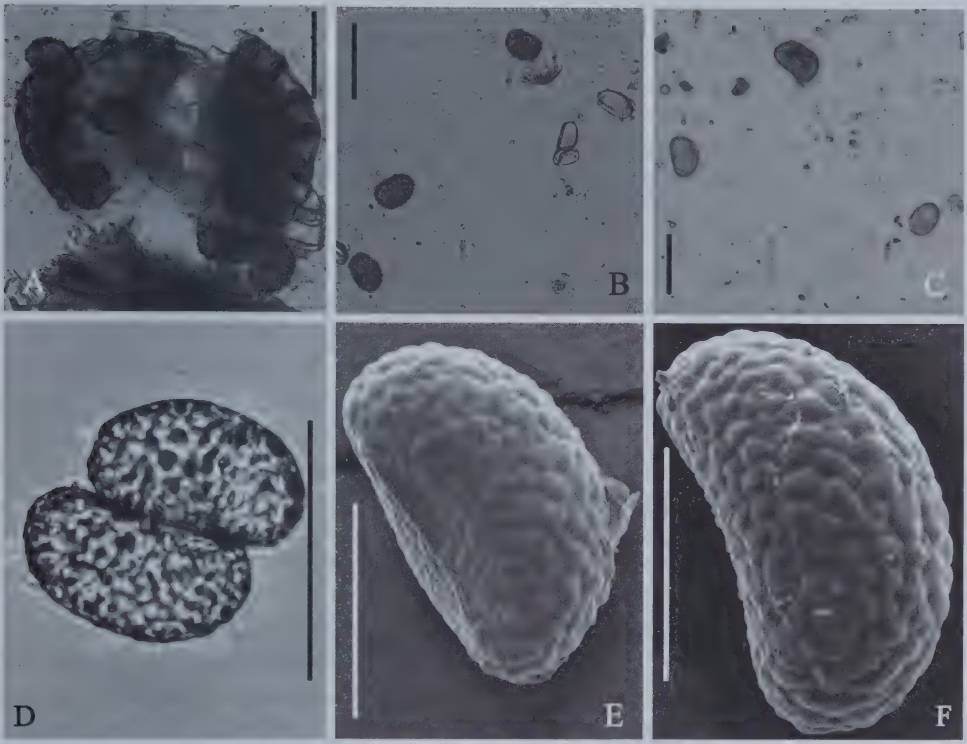

FIG. 2. Sporangium and spores from feces of *Xenotrachea* sp. and *Polypodiodes amoena* under optical microscope and scanning electron microscope. A. Broken sporangium in the feces of *Xenotrachea* sp. under optical microscope; B–C. Spores in the feces of *Xenotrachea* sp. under optical microscope; D. Spores of *Polypodiodes amoena* under optical microscope; E–F. Spores of *Polypodiodes amoema* under scanning electron microscope. Scale bars: A–D, 50 μm, E–F, 20 μm.

larvae on the fern leaves, especially when those leaves showed signs of physical damage (Fig.1 C, D).

Feces produced by the caterpillars were dissolved in water and observed using an optical microscope, revealing the presence of broken sporangia and spores (Fig. 2 A–C) that morphologically corresponded to those of the host plant (Fig. 2 D–F). This reflects the intimate relationship between the caterpillars and *P. amoema*.

The caterpillars were reared in the lab until they formed pupae (Fig.1 E). The color pattern changed with the growth of the larva, corresponding to the patterns observed in the wild larvae. One adult hatched from a pupa after 36 days (Fig. 1 F). The specimen was identified based on the morphological characteristics and DNA barcoding.

One principle of species identification is to compare the male/female genital organs, but our observations were limited because we only had one specimen. Therefore, we identified the specimen to the genus *Xenotrachea* based on the following morphological characteristics: small-to-medium in size, a large white spot surrounded by black spots at the center of the forewing, and a horizontal white spot at the apex of the forewing.

After DNA extraction, a modified PCR protocol for COI amplification (Ivanova, Mol. Ecol. Notes 6:998–1002. 2006) was conducted in volumes of 25 μL containing 0.25 μL *Taq* Polymerase (5000 U/mL, Promega), 2.5 μL 10× PCR buffer (2.0 mM $MgCl_2$), 2 μL dNTPs (10 mM), 1 μL of each primer (10 pmol/lL), 1 μL template DNA and 17.25 μL dH2O. We recovered a COI sequence that was 594bp (GenBank accession number: KR779873). This sequence was submitted to the BOLD Identification System (http://www.boldsystems.org/index.php/IDS_IdentificationRequest) for identification. The result showed that the sequence with the most similar match was *Xenotrachea albidisca pseudodisca*, but a species level match could not be made. So we named it Noctuidae, *Xenotrachea* sp. Our study is the first report of background matching in the genus *Xenotrachea*.

This observation provides new insights into insect–fern interactions and suggests that there may be a significantly larger number of unknown interactions between insects and ferns than recognized. However, currently there is no evidence that this mimesis prevents predation of the caterpillars, nor do we know the possible predators involved.

This work was supported by China Shanghai Landscaping & City Appearance Administrative Bureau Scientific Research Grants F132421 and F112422, and the help of Mr. Hui-Min Chen in Qiyunshan Natural Reserve of Jiangxi, China. The insect was identified by Prof. Ping You from Shaanxi Normal University.—C. X. Wang, and Y. H. Yan, Shanghai Chenshan Plant Science Research Center, the Chinese Academy of Sciences; Chenshan Botanical Garden, Shanghai. E-mail: yhyan@sibs.ac.cn.

American Fern Journal 106(3):227–229 (2016)
Published on 19 October 2016

SHORTER NOTES

Clarifying the taxonomy of Alaskan *Asplenium trichomanes* populations.—Alaska represents the northern extent of *Asplenium trichomanes* L. in North America and the species is known from four localities in the southeastern portion of the state. The Flora of North America lists the diploid subspecies *A. trichomanes* subsp. *trichomanes* L. as the only subspecies occurring in Alaska (Wagner et al., Aspleniaceae, in: Flora of North America Editorial Committee (Eds.), The Flora of North America, Vol. 2. Oxford University Press, New York, NY, pp. 228–245. 1993), although the tetraploid *A. trichomanes* L. subsp. *quadrivalens* D.E. Meyer is more common at higher latitudes (Moran, American Fern Journal 72:5–11. 1982).

The diploid *A. trichomanes* subsp. *trichomanes* is characterized by a strong preference for acidic substrates, in contrast to the calciphile tetraploid *A. trichomanes* L. subsp. *quadrivalens*. However, label data for four specimens deposited at the University of Alaska Museum Herbarium (ALA) identified as *A. trichomanes* were collected from limestone cliffs. Therefore, I tested the hypothesis that Alaskan *A. trichomanes* populations were actually the limestone-loving tetraploid subspecies.

The diploid and tetraploid subspecies are difficult to distinguish morphologically and the acidic substrate preference of the diploid is not absolute (Moran, 1982). Spore size is consistently different, with diploid spores having a mean value of 29.4 µm (ranging from 27–36 µm) and tetraploid spores having a mean value of 41.0 µm (ranging from 34–43 µm) (Erkt and Štech, Preslia 80:325–347. 2008; Moran, 1982). I measured 10–15 spores per specimen for all four Alaskan *A. trichomanes* specimens deposited at ALA, as well as a known tetraploid specimen collected in Scotland. Spores were immersed in water and measured along their longest axis (illustration A2 in Fig. 2, Wagner et al., American Fern Journal 76:129–140. 1986). No diploid specimens were available at ALA, so the previously published mean value and typical size range for diploid *A. trichomanes* subsp. *trichomanes* spores were used for comparison (Moran, 1982).

Spore measurements of Alaskan specimens ranged from an average value of 38.3 µm to 44.5 µm, clearly showing that all four specimens represented tetraploid *A. trichomanes* subsp. *quadrivalens* (Fig. 1). The presence of diploid *A. trichomanes* subsp. *trichomanes* in Alaska is now unconfirmed and the Flora of North America report of its presence (Wagner et al., 1993) is likely in error. However, its presence in British Columbia, Washington, and Oregon, suggest that further collecting efforts could uncover its presence in Alaska as well.

I did not detect any deformed spores that could be indicative of the triploid hybrid between diploid and tetraploid subspecies that has been reported from the eastern United States (Moran, 1982). Calcareous bedrock is commonly encountered throughout southeast Alaska (Wilson et al., Geologic map of

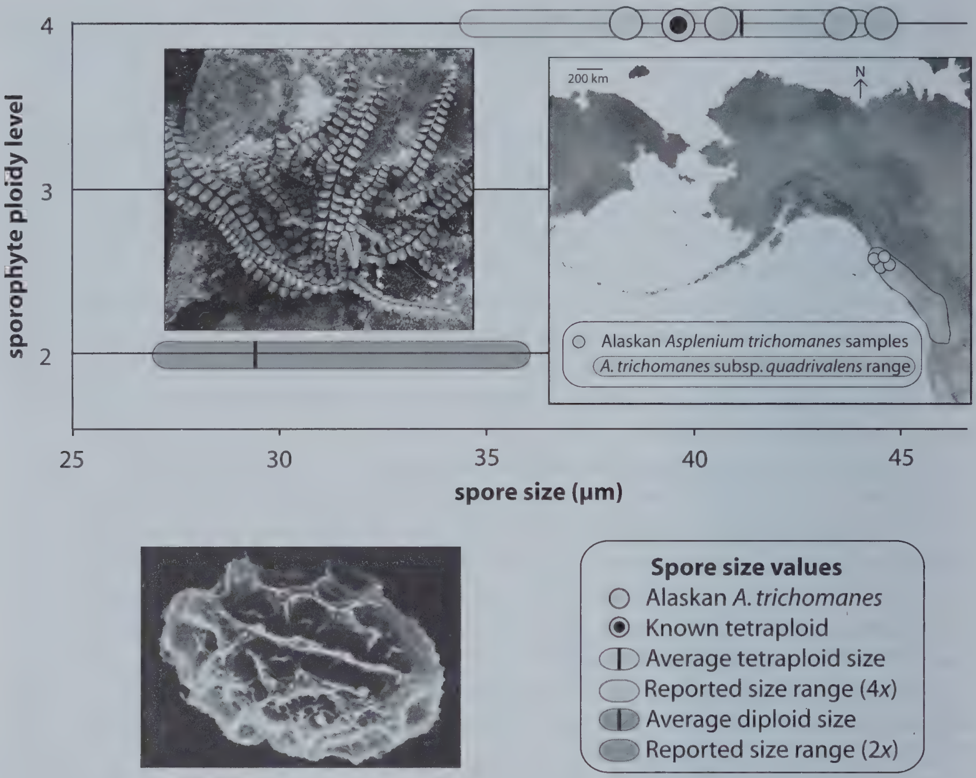

FIG. 1. Variation in spore size between cytotypes in North American *Asplenium trichomanes*. Spore size measurements are plotted against ploidy level. Average and reported values for each subspecies were previously published (Moran, 1982). Photograph of live *A. trichomanes* from Coronation Island, Alaska, is by Karen Dillman. The map depicts the range of *A. trichomanes* subsp. *quadrivalens* in northwestern North America and is based on current specimens examined and previous study (Moran, 1982). The electron micrograph depicts a tetraploid *A. trichomanes* spore (modified from Tryon and Lugardon, Spores of the Pteridophyta. Springer-Verlag, New York, NY. 1991).

Alaska: U.S. Geological Survey Scientific Investigations Map 3340, pamphlet 196. 2015, http://dx.doi.org/10.3133/sim3340), so the northern extent of *A. trichomanes* subsp. *quadrivalens* is likely determined by climatic factors. Photomicrographs of spore measurements and links to specimen database records for all included specimens are available in the Arctos database (http://arctos.database.museum/project/alaskan-asplenium-trichomanes-subspecies-identification-using-spore-measurements).

SPECIMENS EXAMINED.—*Asplenium trichomanes*: USA, Alaska, (1) Coronation Island. David R. Klein *561*, 4 August 1959 (ALA). (2) Dall Island, mouth of bay. Elev. 3 m. Carolyn L. Parker *5165*, 1 July 1994 (ALA). (3) Prince of Wales Island, ¾ mile from Wooden Wheel Cove. Linda Vorobik *190*, 19 July 1979 (ALA). (4) Prince of Wales Island, Moira Sound, Cannery Cove. Elev. 15 m. Mary Stensvold *6187*, 11 August 1993 (ALA).

I thank Stefanie Ickert-Bond for assistance with preparing microscope slides and comments on the manuscript. I thank Karen Dillman for providing a photograph of live *A. trichomanes* from southeast Alaska. I thank Edward Alverson, Robbin Moran, Mary Stensvold, and Weston Testo for helpful discussion.—J. S. METZGAR, Herbarium (ALA), University of Alaska Museum of the North and Dept. of Biology and Wildlife, University of Alaska Fairbanks, Fairbanks, AK 99775, U.S.A., email: jsmetzgar@alaska.edu.

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COVER CAPTION: Asplenium trichomanes growing on a cliff on Coronation Island, Alaska. Photo by Karen Dillman.